Variability of the field potential duration of stem cell-derived cardiomyocytes is a proarrhythmic indicator

A. Bruening-Wright, C.A . Obejero-Paz, J. Kramer, A.M. Brown. Charles River, Cleveland, USA

discovery from charles river

1 INTRODUCTION

The investigation of the effect of drugs on the extracellular field potential (FP) of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) is part of the new Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative promoted by the FDA. The CiPA goal is to develop proarrhythmic markers based on mechanistic understanding of drug-induced cardiac arrhythmias including, but not limited to, Torsade de Pointes (TdP), a self-limited polymorphic tachycardia that can evolve to ventricular fibrillation.

Most of the drugs with TdP liability prolong the cardiac action potential (AP) by blocking the hERG channel. This increases the chances of calcium and sodium current reactivation during the second and third phases of the AP and the generation of early after depolarizations (EADs) and cardiac arrhythmias. AP prolongation underlies prolongation of the Field Potential Duration (FPD) in Multi-Electrode Array (MEA) studies of layers of iPSC-CMs, and underlies prolongation of the QT interval of the ECG.

However, AP prolongation is not always associated with TdP. Studies in dogs (Hondeghem et al. 2001, Thomsen et al., 2004) and cardiac stem cells (Yamazaki et al., 2014) show that increases in beat-to-beat variability are more predictive of TdP than QT interval and FPD prolongation.

Beat-to-beat variability of the of cardiac AP at the single cell level is due to the stochastic behavior of ion channels (Zaniboni et al., 2000; Tankanen and Alvarez, 2007). Electrotonic coupling decreases beat-to-beat variability (Zaniboni et al., 2000), however AP variability still can be observed at the tissue level (Hondeghem et al. 2001, Thomsen et al., 2004). Currently AP variability is characterized using the Poincaré plot descriptors SD1 and SD2 (Brennan et al., 2001) that measure the dispersion of points across and along the identity line. However, this approach does not quantify the extent to which AP variability follows changes in AP duration.

Here we show that the slope of the functional relationship between the coefficient of variation of the FPD (FPD_{CV}) and FPD is a predictive marker of a drug's proarrhythmic potential.

2 METHODS

The Axion Biosystems Maestro instrument was used to record extracellular field potentials. Experiments were performed using Cor.4U cells in serum free medium and at 37°C. Drugs were added in increased concentrations as indicated in the respective sections. The concentration of the vehicle (DMSO) was 0.3 %. The Axion Biosystems software (AXiS) was used to quantify field potential duration (FPD), Na+ spike amplitude, and beating rate. In house algorithms were used to detect proarrhythmic events including EADs and triggered events and to quantify beat-to-beat variability. We measured beat-to-beat variability using Poincaré plots constructed using 30 consecutive FPDs. Short term and long term variability was measured using the plot descriptors SD1 and SD2. We also used FPD_{CV} calculated as (SD/mean x 100) as a measure of beat-to-beat variability. This parameter has a more straightforward interpretation when compared with changes in FPD. Data is presented as mean ± SEM. Here we show data from a single plate. A second plate showed comparable results.

Spontaneously active Cor.4u cells shows transient changes in beat rate that influence the FPD. Figure 1 shows a period and FPD time series to illustrate this relationship. The figure also shows Poincaré plots constructed using 30 consecutive data points from three different regions. Note that the region with the smaller SD1 FPD value (green encircled points) is in the region with the smaller SD1 period value. The Poincaré plots at the right indicate the SD1 FPD values and coefficient of variation for the three conditions. We used the same approach for the different drug treatments: regions with the lowest variability in period for each record were analyzed.

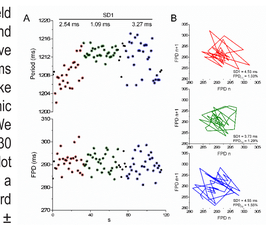


Figure 1: A) Period (top) and FPD (bottom) time series. The SD1 values at the top were calculated from Poincaré plots of the period at each colored region. B) Poincaré plots of FPD values from the three regions. The calculated SD1 and FPD_{CV} values of each region are also included.

3 RESULTS

Effect of tetrodotoxin on FPD and beat-to-beat variability

Beat-to-beat variability of single guinea pig cardiac APs is significantly reduced by 13 µM TTX (Zaniboni et al., 2000). Figure 2 shows that TTX concentrations up to 1 µM, which decrease Vmax of the cardiac action potential by ~40 % (IC_{50} 1.6 µM, not shown), have no effect on FPD and FPD_{CV}. FPDs in TTX showed lower variability with respect to time matched controls, however the differences were not statistically significant and showed no concentration dependence. Minimal electrical activity was recorded at 3 µM TTX. Interestingly, the only well with spontaneous activity at this concentration showed shorter FPDs and decreased variability.

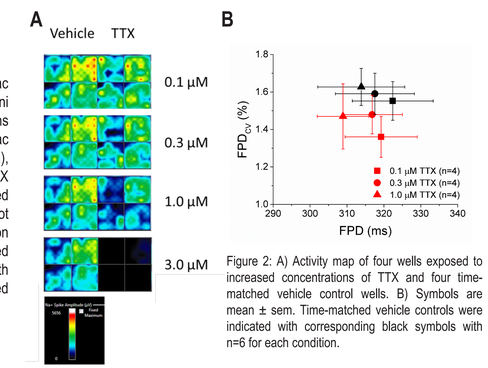


Figure 2: A) Activity map of four wells exposed to increased concentrations of TTX and four time-matched vehicle control wells. B) Symbols are mean ± sem. Time-matched vehicle controls were indicated with corresponding black symbols with n=6 for each condition.

Calcium channel blockers shorten FPD and increase FPD_{CV}

Calcium channel block was associated with a significant concentration dependent reduction of the FPD and increase in FPD_{CV}. While the mechanism is not known, the spontaneous beat rate is increased markedly by calcium block, and it is speculated that part of the increased variability may be explained by a significant reduction of the T wave amplitude as shown in Figure 3B.

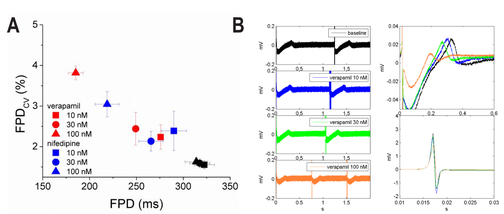


Figure 3: A) Effect of increased concentrations of verapamil and nifedipine on FPD_{CV} and FPD. The slopes of the functional relationship between FPD_{CV} and FPD for verapamil and nifedipine were -0.018 and -0.011 FPD_{CV}%/ms. B) Example of the effect of verapamil on the FP waveform. Left panel: superposition of detected FPs in a 2s display window. Top and bottom right panels: view of the averaged responses focusing on FP repolarization and sodium spike amplitude, respectively.

The slope of the functional relationship between FPD_{CV} and FPD is a proarrhythmic marker independent of the plasma concentration

Figure 4A shows the effect of torsadogenic drugs on the functional relationship between FPD_{CV} and FPD. The relationship was quantified by the slope shown in the legend. Except for moxifloxacin that shows a very shallow slope, all torsadogenic drugs showed steeper slopes. Figure 4B shows examples of the FPDs in vehicle, and moxifloxacin and dofetilide with comparable FPD prolongations. Note that T wave peaks are well defined in control and moxifloxacin and less defined in dofetilide, a consequence of a flattening of the T wave and dispersion of repolarization. Also note that the increased variability is not due to the presence of triggered activity.

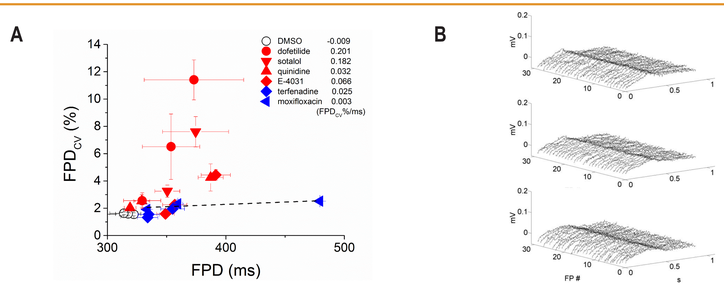
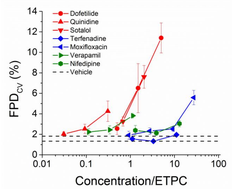


Figure 4: A) Functional relationship between FPD_{CV} and FPD. Symbols indicate mean ± SEM of at least three wells. Numbers in the legend indicate the slope of the functional relationship between FPD_{CV} and FPD. For each drug, concentrations were increased in an stepwise manner. Only concentrations with no arrhythmic activity were included in the analysis. The data represents activity recorded in 1, 3 and 10 nM dofetilide; 10 and 30 µM sotalol; 0.1, 0.3 and 1 µM quinidine; 1, 3 and 10 nM E-4031; 10, 30 and 100 nM terfenadine and 10, 30, 100 and 300 µM moxifloxacin. The dashed line is the linear fit to the moxifloxacin data. Red symbols indicate torsadogenic drugs. Blue symbols indicate torsagogenic drugs only at higher concentrations or overdoses. B) 3D plots constructed with 30 consecutive FPs to show the different T wave morphology. Records are from electrodes with FPD_{CV} values close to the mean value.

FPD_{CV} *per se* is not a pro-arrhythmic marker

A plot of FPD_{CV} as a function of the plasma concentration in Figure 5 reveals that safe drugs like verapamil and nifedipine show high FPD_{CV} values compared to vehicle, whereas a torsadogenic drug like terfenadine shows FPD_{CV} values comparable to control. Interestingly moxifloxacin, a drug used as a positive control for QT prolongation, show FPD_{CV} values comparable to nifedipine. Large FPD_{CV} values for moxifloxacin were observed only at plasma concentrations 30 times larger than the values observed in the clinic.

Figure 5: Functional relationship between FPD_{CV} and the Effective Therapeutic Plasma Concentration (ETPC). Dashed lines indicate the minimum and maximum 95% confidence intervals measured in vehicle controls. Note that non-TdP drugs like verapamil and nifedipine show FPD_{CV} values above controls and that the TDP drug terfenadine shows values comparable to controls. Drugs shown in red are torsadogenic, green are non-torsadogenic and blue are only torsadogenic at higher concentrations or overdoses.



4 CONCLUSIONS

1) Beat-to-beat variability of the FPD is an intrinsic property of hiPSC-CMs that in part is associated with changes in beating frequency.
2) Whether sodium-dependent variability is present in hiPSC-CMs remains to be confirmed.
3) Shortening of the action potential by calcium channel blockers is associated with increased variability and underlies a negative slope of the functional relationship between FPD_{CV} and FPD.
4)Torsadogenic drugs induced steep changes in beat-to-beat variability. This effect was not due to EADs.
5) Moxifloxacin, a torsadonenic drug only at high plasma concentrations, shows a shallow slope consistent with its safety profile in the clinic.
6) The slope of the functional relationship between FPD_{CV} and FPD is a novel proarrhythmic marker for TdP that is independent of the plasma concentration.

REFERENCES
Zaniboni et al., 2000. Am. J. Physiol. Heart Circ. Physiol. 278:H677.
Yamazaki et al., 2014. Pharmacol Pharm. 5:117.
Brennan et al., 2001. IEEE Trans. Biomed Eng.48:1251.
Hondeghem et al., 2001. Circulation 103:2004.
Thomsen et al., 2004. Circulation 110:2453.
Tanskanen and Alvarez, 2007. Math. Biosci. 208:125.

charles river