Determination of Pro-arrhythmic Effects of Compounds in Human iPSC-Derived Cardiomyocytes Using FDSS/µCell Imaging Platform

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Abstract

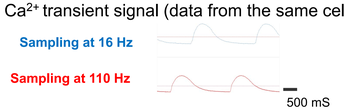
FDSS/µCell is a high-speed acquisition imaging platform (Hamamatsu Ltd., Japan) that allows for simultaneous high-throughput reading under controlled conditions. We evaluated the Ca^{2+} transients or optical membrane potential changes of hiPSC-CMs (iCells®) in the presence or absence of pharmacological agents known to interfere with cardiac ion channels (e.g. hERG, IKs, Nav1.5, Cav1.2). Ca^{2+}-sensitive fluorescence dyes (Codex ACTOne® and EarlyTox®) and a membrane potential dye (FLIPR MP®) were tested. We were able to detect acute and delayed drug effects, quantify and report drug-induced early-after depolarizations (EAD)-like waveforms, ectopic cardiomyocyte beats and changes in beating rate, from a variety of agents. Cardiovascular drugs, such as dofetilide and D,L-sotalol, exhibited EAD-like signals at 3nM and 10µM, respectively. CNS drugs, such as haloperidol and sertindole, exhibited EAD-like signals and ectopic beats at 30nM and 1µM, respectively. Other drugs, such as astemizole, solifenacin, and moxifloxacin, exhibited similar arrhythmias at 30nM, 3µM, and 300µM, respectively. Our data suggest that the membrane potential and intracellular Ca^{2+} signal are tightly coupled, supporting the idea that the EAD-like signals reported are the accurate representation of an EAD signal of the cardiac action potential. Finally, the EAD Ca^{2+} signal was well correlated to reported clinical TdP arrhythmias at relevant concentrations.

Methods and Validation

FDSS imaging platform:

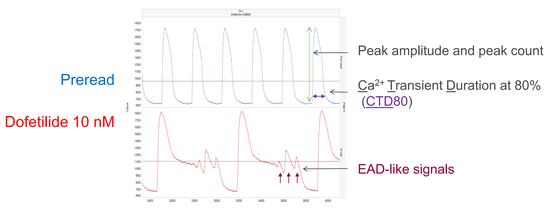
1. Intracellular calcium transient (Ca^{2+} sensitive dye)
2. Membrane potential optical signal (membrane potential dye)

110 Hz high-speed data acquisition mode demonstrated clean Ca^{2+} transient signal (data from the same cells)



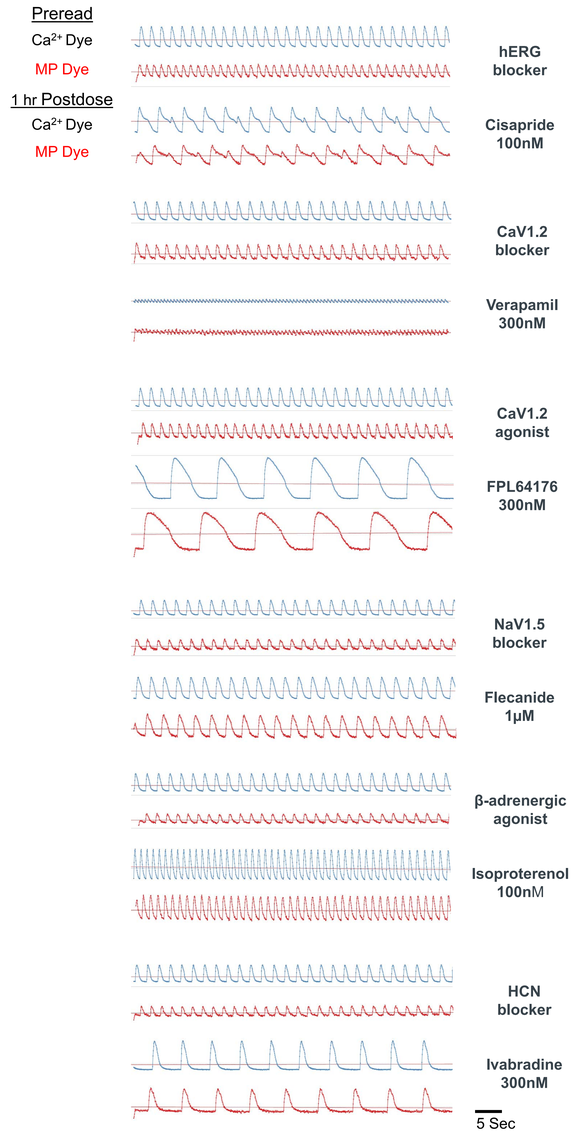
Measurement of endpoints:

1. Peak amplitude and beating rate (peak count)
2. Calcium transient duration (CTD80)
3. EAD-like signals



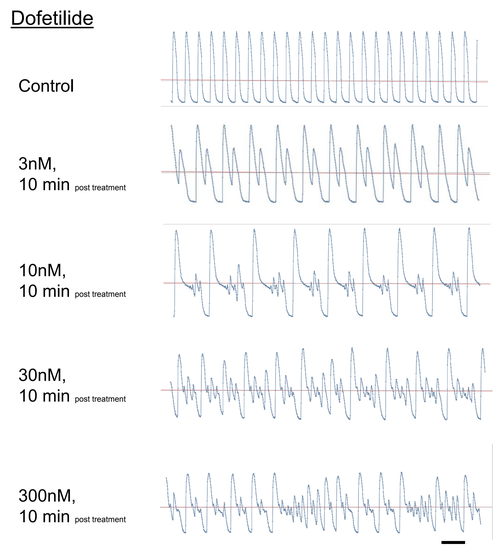
Ca^{2+} and MP Signal Correlation

Ca^{2+} transient signal correlates with membrane potential (MP) signal from the same iCells®



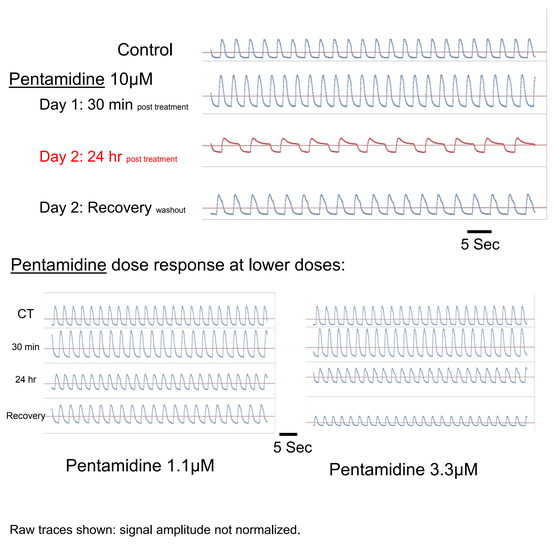
Raw traces shown: signal amplitude not normalized.

Detection of Acute Effects



Raw traces shown: signal amplitude not normalized.

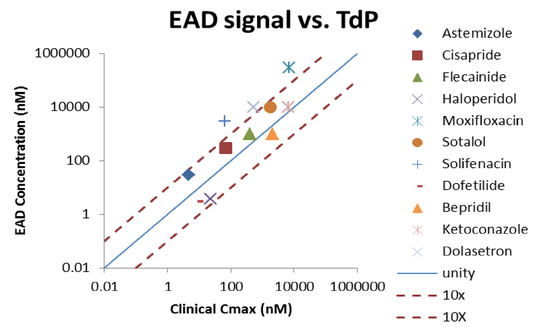
Detection of Delayed Effects



Raw traces shown: signal amplitude not normalized.

Clinical Correlation

EAD conc. vs Clinical TdP concentrations (with available clinical total concentrations)



Good quantitative correlation
for true positives with reported clinical TdP concentrations

Conclusions

- Simultaneous high-throughput reading of Ca^{2+} and membrane potential (MP) signals from iPSC-CMs confirms that both signals are tightly coupled. The EAD signal reported with some compounds represents an EAD of the action potential caused by the effects of the drug.
  - Cisapride (Ikr blocker) is an example of a compound that exhibited EADs when the MP dye was used simultaneously with the Ca^{2+} dye (experiments recorded from the same cells).
  - Similar signal patterns are reported for the other compounds that did not exhibited EADs but changes in beat rate and amplitude.
- Acute effects: Dofetilide, known to be torsadogenic in the clinic (Ikr blocker), exhibited EADs after 10 min incubation period, with pronounced anti-arrhythmic patterns exhibited at higher doses.
- Delayed effects (e.g., after 24 hrs of drug incubation time): 10µM pentamidine (disrupts hERG protein trafficking) exhibited no changes after 30-min incubation. After 24 hrs. post-treatment, EADs were detected (baseline effect was recoverable after a washout period). Cardiac arrhythmias have been reported in patients taken pentamidine over time (Lidman et al, 1994).
- In all standards tested, the EAD Ca^{2+} signal was well correlated to reported clinical TdP at relevant concentrations.
- Overall, by monitoring Ca^{2+} transient parameters and the morphology of the Ca^{2+} and MP signal that elucidate EADs when there is potential for arrhythmias, we were able to predict the proarrhythmic cardiac effect of a variety of drugs.