Comparison of Media Effects on Cardiac Safety Testing using induced Pluripotent Stem Cell Derived Cardiomyocytes

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Background

- In a concerted international effort, the regulatory paradigm for assessing cardiac safety is being reshaped into the Comprehensive In-vitro Proarrhythmia Assay (CiPA).
- A core component of CiPA will be the application of induced pluripotent stem cell-derived cardiomyocyte (iPSC-CM) electrophysiology measurements to supplement thorough ion-channel patch clamp assessment and in-silico modelling [1].
- There exists a variety of protocols and methods appropriate for proarrhythmia testing using iPSC-CMs encompassing multiple assays and platforms as well as materials.
- For optical electrophysiology assays, including voltage sensitive dyes and calcium imaging, the experimental culture media needs to be free of serum to minimize scattering effects; micro-electrode array (MEA) testing allows for the use of either serum-containing or serum-free media formulations.
- Media free of serum has emerged as the gold standard for drug testing as it minimizes drug-protein binding interactions.
- Here we investigate potential differences in drug effects depending on media formulation as observed in MEA measurements. 25 drugs were tested on iPSC-CMs in both serum-containing and serum-free media. The serum-free results have previously been analyzed and published [2].

Methods

- Human iPS-cardiomyocytes (iCell Cardiomyocytes, Cellular Dynamics International) were thawed and plated according to the manufacturer's instructions onto 48-well MEA plates (Axion BioSystems, M768-KAP-48) coated with droplets of fibronectin.
- Cell culture was maintained at 37ºC and 5% CO_2, with media changes occurring every 48h.
- MEA assaying was performed 10-14 days post-thaw using a Maestro MEA platform (Axion Biosystems).
- Two hours before baseline recordings, half of the MEA plates were switched from serum-containing maintenance media (CDI, CMM-100-120-005) to serum-free DMEM (Corning, 17-205-CV).
- Of the media formulations, the differences in composition include the addition of 10% fetal bovine serum in the maintenance media, as well as the inclusion of galactose rather than glucose to serve as the carbon substrate.
- Four doses of each drug were administered using sequential dosing in three replicate wells.
- Drug dilutions were prepared via serial dilution of the stock in both media formulations. Drug stocks prepared at 10 mM (or 200 mM for Moxifloxacin) in DMSO or water depending on solubility information provided by the manufacturer.
- Blinded data analysis was conducted offline using AXiS software (Axion Biosystems).

Results: FPDc and Arrhythmia Response

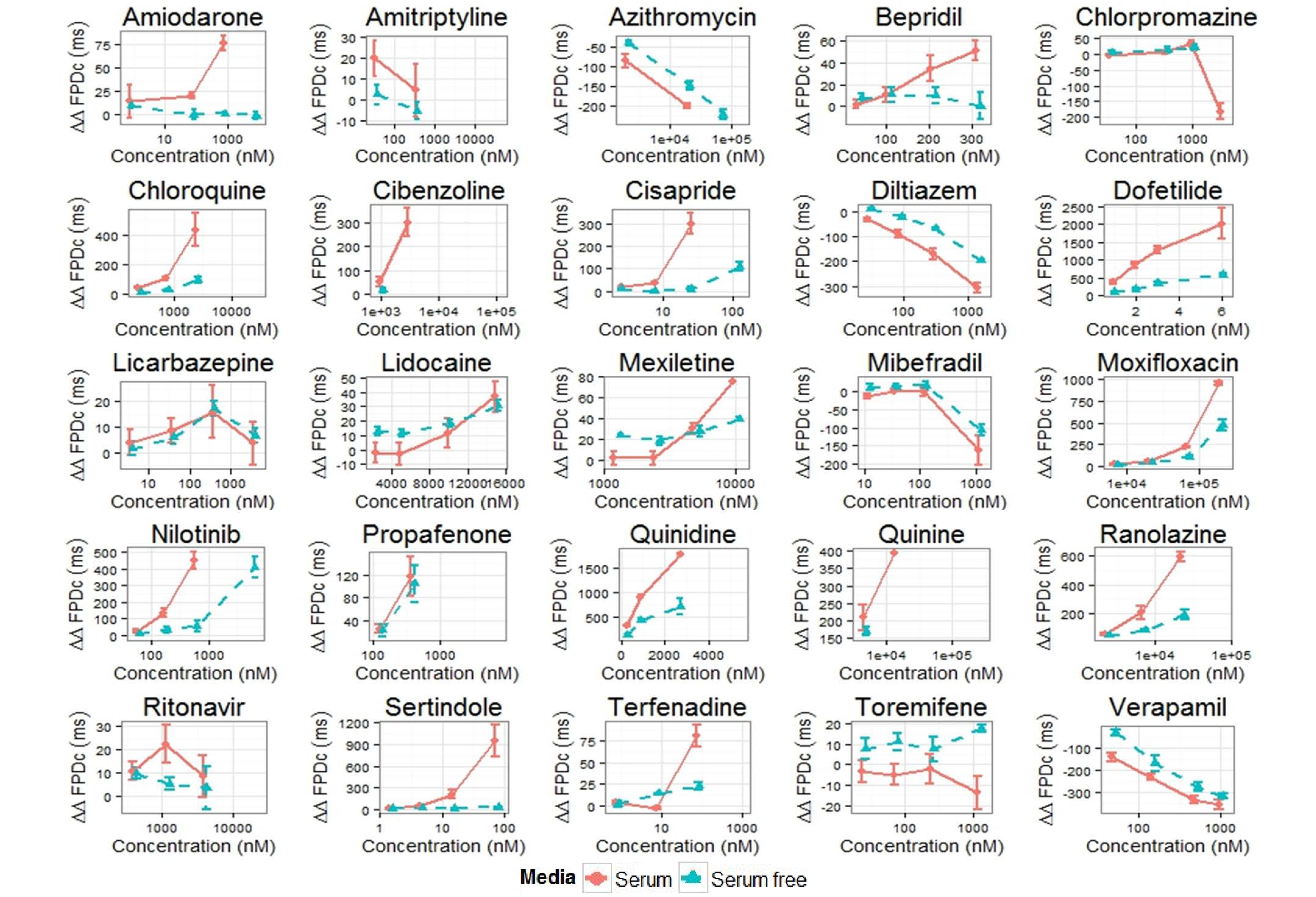

Figure 1: Dose response curves for all 25 drugs showing the drug induced change in FPDc from baseline and vehicle controls in both serum-containing maintenance media and serum-free DMEM. Quiescent wells were removed. Each drug was applied to three wells, except Dofetilide, which was dosed into 9 wells.

Number of Arrhythmic and Quiescent wells in Serum-Containing & Serum-Free Media

<table>
<tr><th></th><th>Dose 1</th><th>Dose 2</th><th>Dose 3</th><th>Dose 4</th></tr>
<tr><td>Amiodarone</td><td>0|0</td><td>0|0</td><td>0|0</td><td>3|0</td></tr>
<tr><td>Amitriptyline</td><td>0|0</td><td>0|0</td><td>3|3</td><td>3|3</td></tr>
<tr><td>Azithromycin</td><td>0|0</td><td>0|0</td><td>2|1</td><td>3|3</td></tr>
<tr><td>Bepridil</td><td>0|0</td><td>0|0</td><td>0|0</td><td>0|0</td></tr>
<tr><td>Chloroquine</td><td>0|0</td><td>0|0</td><td>3|0</td><td>3|3</td></tr>
<tr><td>Chlorpromazine</td><td>0|0</td><td>0|0</td><td>0|0</td><td>2|1</td></tr>
<tr><td>Cibenzoline</td><td>0|1</td><td>3|3</td><td>3|3</td><td>3|3</td></tr>
<tr><td>Cisapride</td><td>0|0</td><td>0|0</td><td>3|0</td><td>3|0</td></tr>
<tr><td>Diltiazem</td><td>0|0</td><td>0|0</td><td>0|0</td><td>0|0</td></tr>
<tr><td>Dofetilide</td><td>0|0</td><td>7|0</td><td>9|4</td><td>9|9</td></tr>
<tr><td>Licarbazepine</td><td>0|0</td><td>0|0</td><td>0|0</td><td>0|0</td></tr>
<tr><td>Lidocaine</td><td>0|0</td><td>0|0</td><td>0|0</td><td>0|0</td></tr>
<tr><td>Mexilitene</td><td>0|0</td><td>0|0</td><td>0|0</td><td>2|1</td></tr>
<tr><td>Mibefridil</td><td>0|0</td><td>0|0</td><td>0|0</td><td>0|0</td></tr>
<tr><td>Moxifloxacin</td><td>0|0</td><td>0|0</td><td>0|0</td><td>3|2</td></tr>
<tr><td>Nilotinib</td><td>0|0</td><td>0|0</td><td>1|0</td><td>3|3</td></tr>
<tr><td>Propafenone</td><td>0|0</td><td>0|0</td><td>3|3</td><td>3|3</td></tr>
<tr><td>Quinidine</td><td>3|0</td><td>3|3</td><td>3|3</td><td>3|3</td></tr>
<tr><td>Quinine</td><td>3|0</td><td>3|3</td><td>3|3</td><td>3|3</td></tr>
<tr><td>Ranolazine</td><td>0|0</td><td>0|0</td><td>2|1</td><td>3|2</td></tr>
<tr><td>Ritonavir</td><td>0|0</td><td>0|0</td><td>0|0</td><td>2|1</td></tr>
<tr><td>Sertindole</td><td>0|0</td><td>0|0</td><td>1|0</td><td>3|0</td></tr>
<tr><td>Terfenadine</td><td>0|0</td><td>0|0</td><td>0|0</td><td>3|1</td></tr>
<tr><td>Toremefine</td><td>0|0</td><td>0|0</td><td>0|0</td><td>0|0</td></tr>
<tr><td>Verapamil</td><td>0|0</td><td>0|0</td><td>0|0</td><td>0|0</td></tr>
</table>

Table 1: Arrhythmic events and quiescence tabulated as number of arrhythmic and quiescent wells in Serum|Serum Free experimental conditions. Red doses indicate events triggered at a lower dose in serum containing media. Green doses indicate triggered events were the same dose at onset in both media.

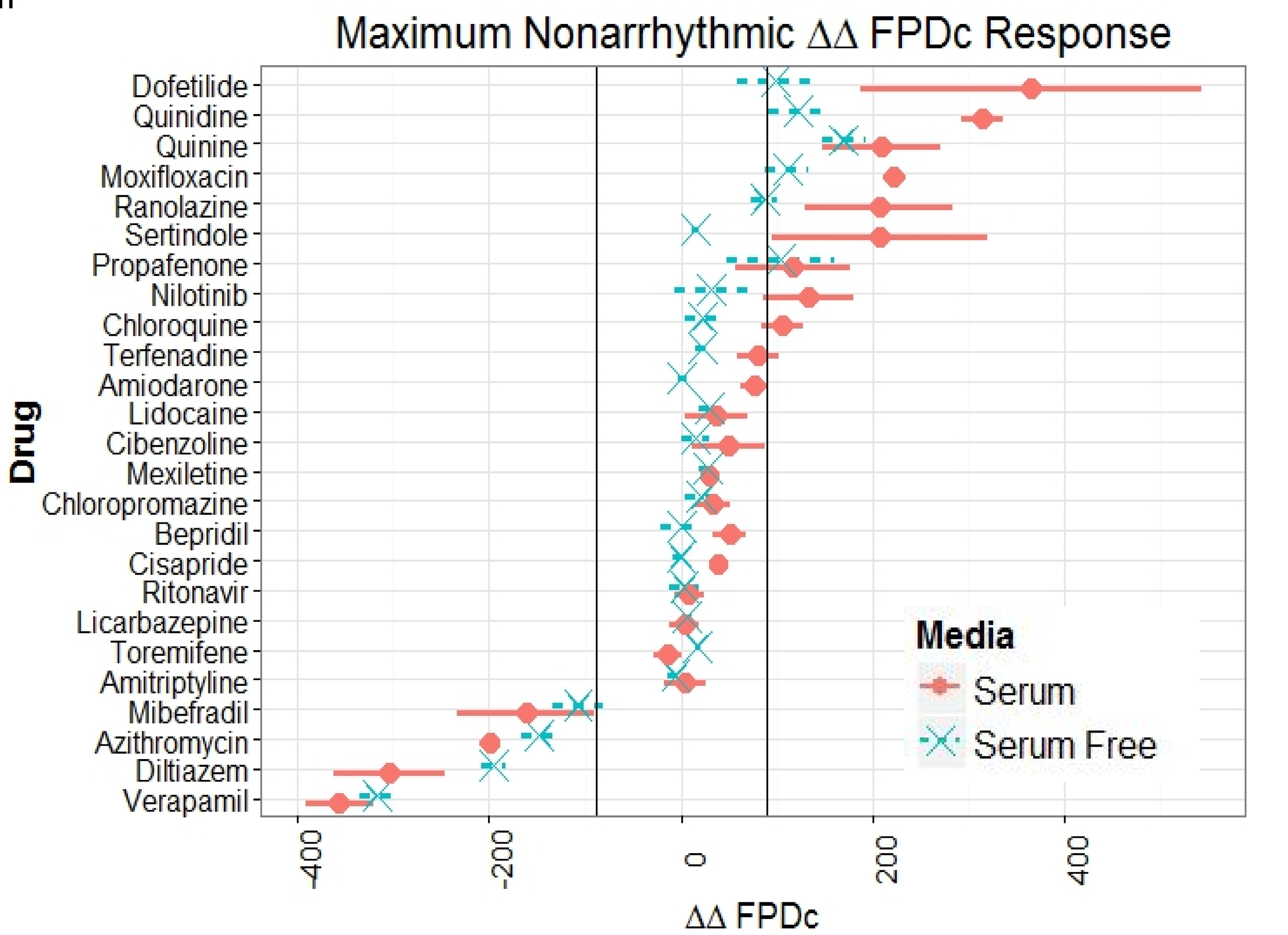

Figure 2: The mean ΔΔFPDc response ± one standard deviation observed at the highest nonarrhythmic or nonquiescent dose. If arrhythmias or quiescence were present in all doses, then ΔΔFPDc was taken from the first dose. Black lines indicate two standard deviations above/below baseline FPDc in DMEM. These serve as thresholds for significant prolongation.

Results: Ratio

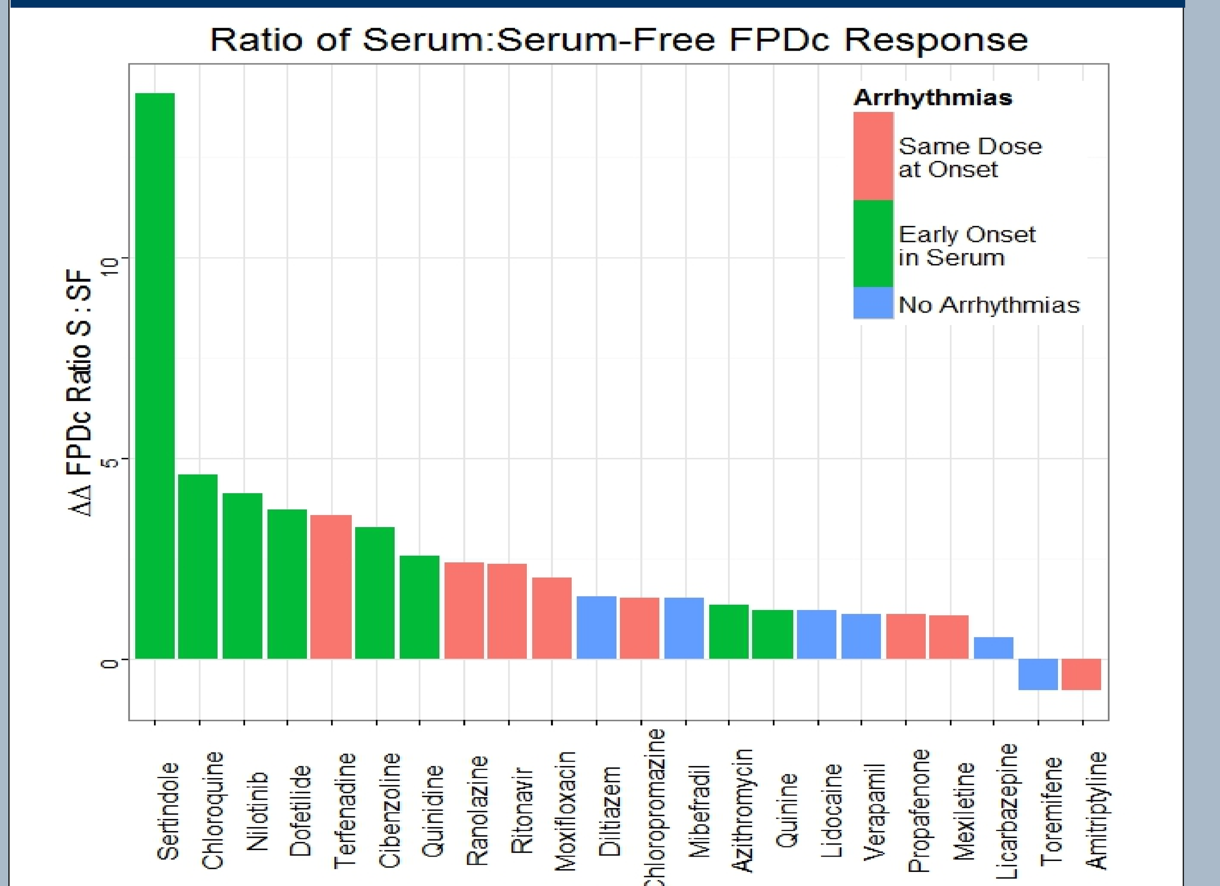

Figure 3: Ratio of serum to serum free maximum nonarrhythmic ΔΔFPDc responses, also represented in Figure 2. Amiodarone, Bepridil, and Cisapride were removed from this plot and had ratios of 62:1, 110:1, and 417:1 respectively.

Results: Solubility of two example drugs

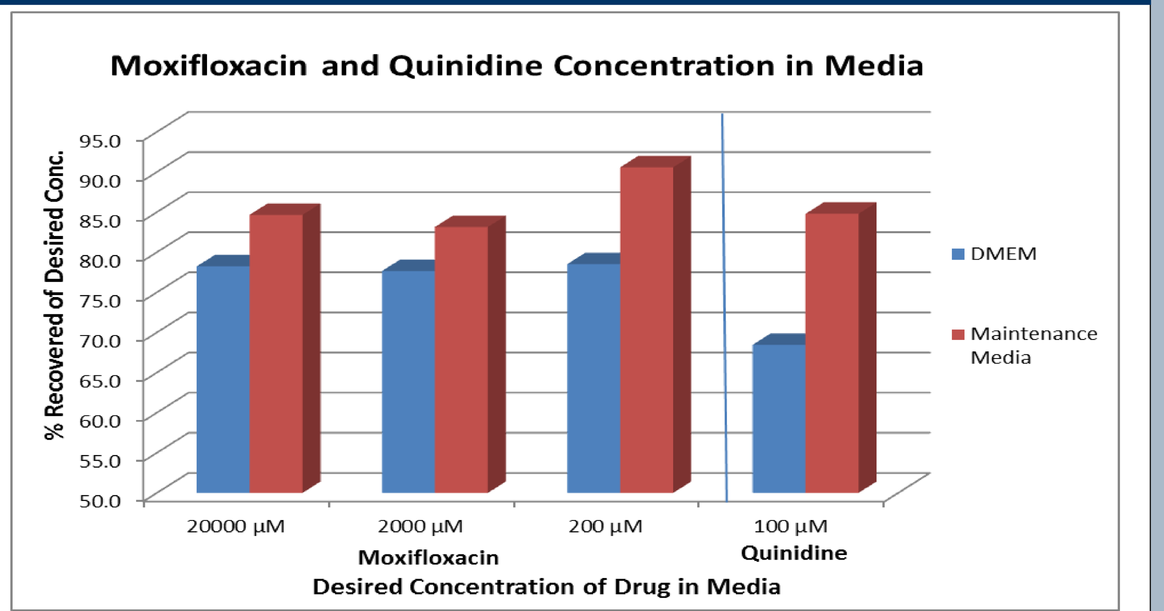

Figure 4: Percent of desired drug concentration detected in media by LC-MS/MS methodologies for a subset of two test drugs in serum-free DMEM and serum-containing maintenance media. Tested samples were prepared using the same serial dilution method used to prepare experimental doses. The persistent trend represents more dissolved drug in serum-containing media.

Conclusions

- Serum-containing media corresponded with higher magnitudes of drug induced FPDc effects and arrhythmic events/quiescence triggered at lower doses.
- Drug concentration analysis for two sample drug dilutions suggests that serum-containing media may have improved compound solubility, consistent with observations suggesting increased drug exposure compared to serum-free media.
- Further research is needed to evaluate and establish consistent best practices in iPSC-CM cardiac safety testing.

References

1. Colatsky, T., et al., *The Comprehensive in Vitro Proarrhythmia Assay (CiPA) initiative — Update on progress.* Journal of Pharmacological and Toxicological Methods, 2016. 81: p. 15-20.
2. Blinova, K., et al., *Comprehensive Translational Assessment of Human Induced Pluripotent Stem Cell Derived Cardiomyocytes for Evaluating Drug-Induced Arrhythmias.* Toxicological Sciences, 2016.

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