Action Potential Recording and Pro-arrhythmia Risk Analysis in Human Ventricular Trabeculae

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Introduction

To assess drug-induced pro-arrhythmic risk, especially Torsades de Pointe (TdP), new models have been proposed, such as in-silico modeling of ventricular action potential (AP) and stem cell-derived cardiomyocytes (SC-CMs). Previously we evaluated the electrophysiological profile of 15 reference drugs in hESC-CMs and hiPSC-CMs for their effects on intracellular AP and extracellular field potential, respectively. Our findings indicated that SC-CMs exhibited immature phenotype and had the propensity to generate false positives in predicting TdP risk.

To expand our knowledge with mature human cardiac tissues for drug-induced pro-arrhythmic risk assessment, human ventricular trabeculae (hVT) from ethically consented organ donors were used to evaluate the effects of the same 15 drugs (8 torsadogenic, 5 non-torsadogenic, and 2 discovery molecules) on AP parameters at 1 and 2 Hz. Each drug was tested blindly with 4 concentrations in duplicate trabeculae from 2 hearts. To identify the pro-arrhythmic risk of each drug, a pro-arrhythmic score was calculated as the weighted sum of percent drug-induced changes in various AP parameters, including AP duration and recognized pro-arrhythmia predictors such as triangulation, beat-to-beat variability and incidence of early-afterdepolarizations, at each concentration. In addition, to understand the translation of this preclinical human AP-based model to clinical studies, a ratio that relates each testing concentration to the human therapeutic unbound Cmax (Cmax) was calculated. At a ratio of 10, for the 8 torsadogenic drugs, 7 were correctly identified by the pro-arrhythmic score; 1 was mislabeled. For the 5 non-torsadogenic drugs, 4 were correctly identified as safe; 1 was mislabeled. Calculation of sensitivity, specificity, positive predictive value, and negative predictive value indicated an excellent performance. For example, at a ratio of 10, scores for sensitivity, specificity, positive predictive value and negative predictive values were 0.88, 0.8, 0.88 and 0.8, respectively. Thus, the human AP-based model combined with the integrated analysis of pro-arrhythmic score can differentiate between torsadogenic and non-torsadogenic drugs, and has a greater predictive performance when compared to human SC-CM models.

Methods

1. Donor heart procurement

All human hearts that were used for this study were obtained by legal consent from organ donors in the US. Policies for donor screening and consent are the ones established by the United Network for Organ Sharing. Organizations supplying human tissues to ANABIOS follow the standards and procedures established by the US Centers for Disease Control and are inspected biannually by the Department of Health and Human Services. Tissue distribution is governed by internal IRB procedures and compliance with HIPAA regulations regarding patient privacy. All transfers of donor organs to ANABIOS are fully traceable and periodically reviewed by US Federal authorities.

2. Recording of action potentials in human ventricular trabeculae

2.1. Tissue dissection: the procedures of tissue dissection and recording were similar to what had been previously described (Page et al., 2016). Briefly, the human heart was transferred into a dissection vessel containing a cold (4oC), fresh proprietary dissection solution. Heart was maintained completely submerged in dissection solution. Ventricular trabeculae were dissected and transferred to the recording chamber.

2.2. Recording of action potentials: a single tissue was mounted into the experimental chamber filled with oxygenated Tyrode's external solution. The temperature of the solution was maintained at 37°C with flow rate at 5 mL per minute. The tissue was allowed to equilibrate for 30 to 60 minutes with stimulation (3V, 3ms) at a frequency of 1.0 Hz. High impedance borosilicate microelectrodes were prepared with a tip resistance of 10 - 20MΩ once filled with 3M KCl. Upon tissue impalement, the membrane potential was allowed to stabilize (typically, around -85 mV). Bipolar stimulation at 1.5x threshold was applied and recordings were performed in continuous mode with sampling at 20 kHz using ADInstruments and LabChart Software.

2.3. Tissue exclusion Criteria: 1).Interruption of perfusion/oxygenation; 2). Absence of APs following stimulation at baseline; 3). Time frame of drug exposure not respected; 4). Unstable response to stimulation at baseline; 5). RMP > -75 mV; 7). AMAX < 70 mV; 8). APD90 < 200 ms or >450 ms.

2.4. Experimental Procedure: Each test article was evaluated at 4 concentrations in 4 ventricular trabeculae derived from a minimum of 2 donor hearts. All tissues tested respected the treatment sequence and time course designated in Figure 1. Briefly, following stabilization of each tissue, APs were collected and assessed for 31 min in vehicle control solution (Tyrode with 0.1% DMSO) at stimulation frequencies of 1 Hz for 25 min, 2 Hz for 3 min and then 1 Hz for 3 min. Following this vehicle control period, 4 concentrations of a test compound were applied sequentially and cumulatively. Each concentration was applied for 31 min with the same stimulation sequence as in vehicle controls

3. Data Analysis

For each frequency tested, the last 30 APs acquired at the end of the period were averaged for vehicle controls and for each test article concentration. Analysis at 1 Hz included only the last 30 APs of the initial 25-min incubation period. The AP parameters and pro-arrhythmia variables as shown in Table 1 were analyzed off-line upon the completion of recordings.

AP parameters and pro-arrhythmia variables were combined into a meaningful, single score to assess the pro-arrhythmic risk of a compound at each concentration tested. The pro-arrhythmic potential of compounds at 1 or 2 Hz was determined by assigning a weighted scale to each variable. The maximum score calculated at either 1 or 2 Hz was selected as The Pro-Arrhythmic Potential Score for each concentration. Based on historical data of this APD assay, a score ≤ 10 indicates a non-pro-arrhythmic potential, while a score ≥ 10 indicates pro-arrhythmic potential.

Reference:

Page et al., (2016) Human ex-vivo action potential model for pro-arrhythmia risk assessment. JPTM 81: 183-195.

Figure 1. Experimental Procedure

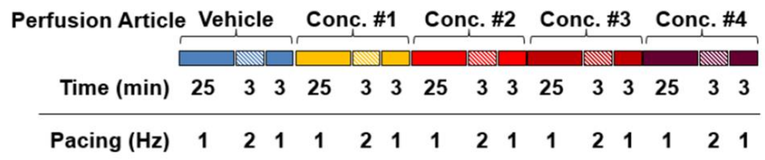

Figure 2. AP traces from hVT in Control and in the Presence of Increasing Concentrations of Tolterodine and Terodiline

Figure 3. Concentration-dependent Changes of APD in the Presence of Tolterodine and Terodiline

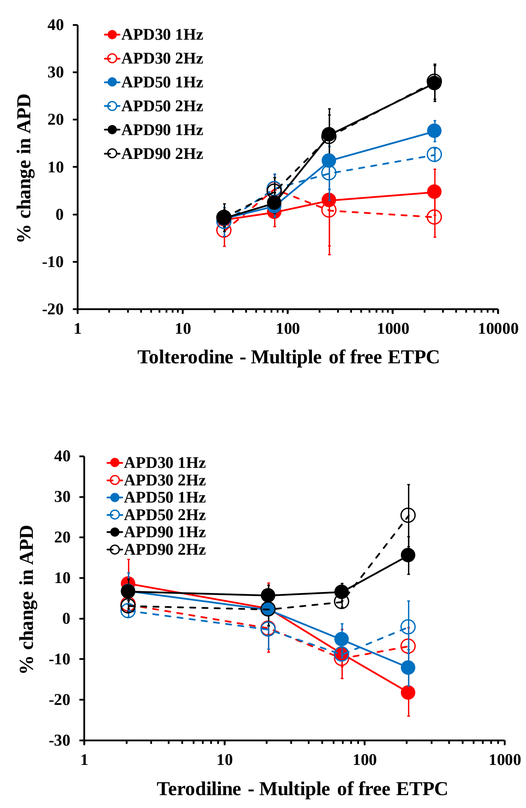

Figure 4. Poincaré Plots of APD90 in the Presence of Tolterodine and Terodiline under 2 Hz Stimulation

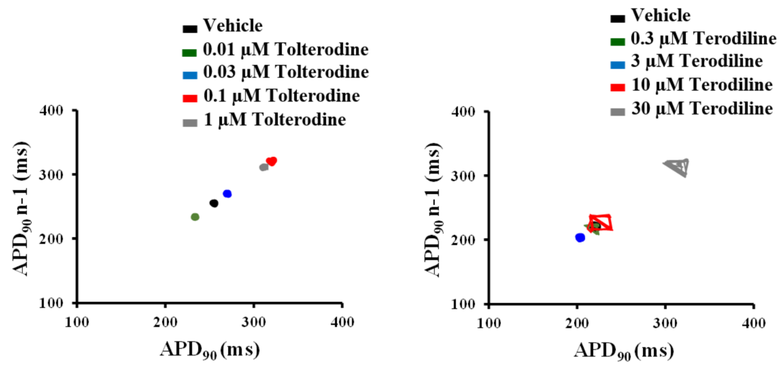

Figure 5. Concentration-dependent Plots of Changes in APD90, Triangulation, STV, and EAD Incidence in the Presence of Tolterodine and Terodiline

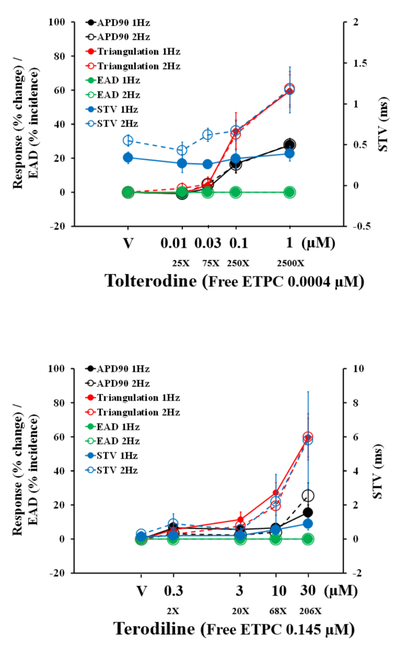

Figure 6. Effects of Tolterodine and Terodiline on STV and triangulation as a Function of APD90 Change

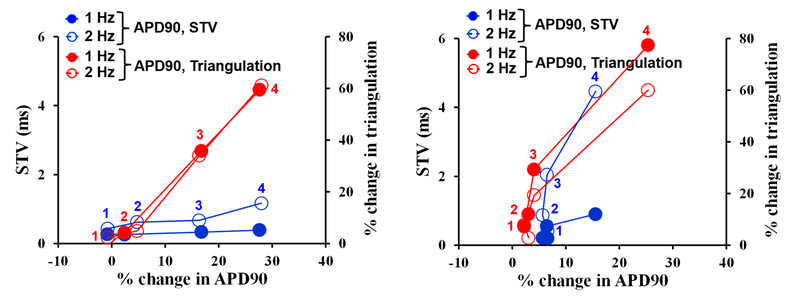

Table 1. Plots of Pro-arrhythmic Score for Tolterodine and Terodiline against Multiples of Free ETPC

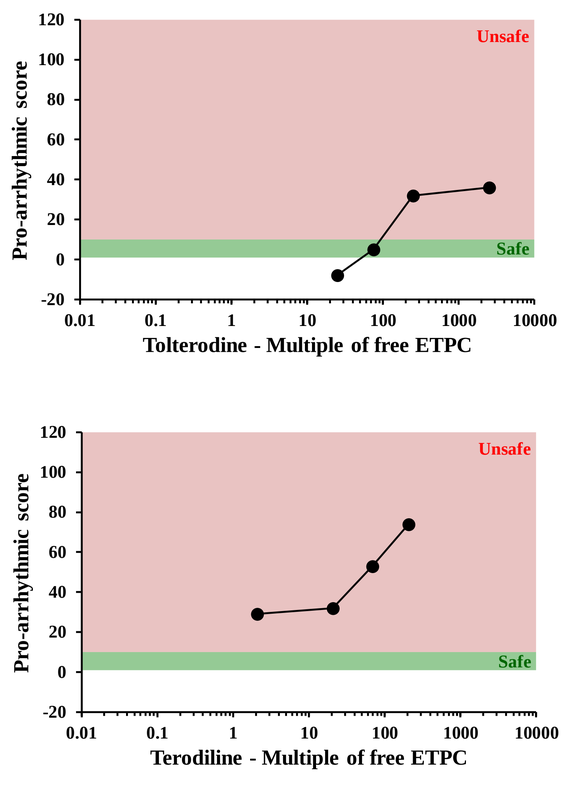

Table 1. Assay Performance of hVT: Comparison with iPSC-CM

		iPSC-CM	iPSC-CM	hVT
		Field Potential Duration	Early After Depolarization	Arrythmic Score
	TdP, Human	10X	10X	10X
Sertindole				
Dofetilide				
Cisapride				
Terfenadine				
Terodiline				
Sotalol (D,L)				
Moxifloxacin				
Flecainide				
Mexiletine				
Tolterodine				
Alfuzosin				
Ranolazine				
Lamotrigine				
Sensitivity		0.88	0.38	0.88
Specificity		0.6	1	0.8
Positive Predictive Value		0.78	1	0.88
Negative Predictive Value		0.75	0.5	0.8

Summary and Conclusions

- Multiple compounds tested blindly in authentic human ventricular tissue for their effects on AP
  - Pro-arrhythmic scores were determined by calculating the weighted sum of drug-induced changes in various AP parameters and pro-arrhythmia variables.
    - The performance characteristics of mature ventricular tissue shown here clearly surpass the reliability of iPSC-CM for pro-arrhythmia detection
- Use of primary human cardiac tissues to evaluate pro-arrhythmia risk in vitro
  - Avoid the confounding influences of the embryonic ion channel expression and spontaneous beat rate observed with hSC-CM
  - Enable a robust and definitive electrophysiological evaluation in mature ventricular myocytes
- Employing primary mature human cardiac tissues for cardiac safety assessment.
  - Not a good screening tool
    - Low throughput nature of the assay
    - The requirement for large numbers of human hearts
  - Placing this model later in the drug development process, i.e., use only for secondary or supplemental purposes