Adult Human Primary Cardiomyocyte Model for the Simultaneous Prediction of Drug-induced Inotropic and Pro-arrhythmia Risk

Nathalie Nguyen, William Nguyen, Brynna Nguyenton, Phachareeya Ratchada, Ky Truong, Guy Page, Paul E Miller, Andre Ghetti and Najah Abi-Gerges

AnaBios Corporation, San Diego, CA 92109, USA

Contact email: **Najah.abigerges@anabios.com**

AnaBios
Early Human Insights

Introduction

The consequence of drug-induced irregular heart beat (pro-arrhythmia) and/or changes in contractility (inotropic liability) can limit the utility of potential novel therapeutics. Since abnormal ventricular repolarization can cause not only electrical disorders (pro-arrhythmia), but also affect the heart's contractile function, the main motivation of this investigation was to develop a human cardiomyocyte-based model that uses adult human primary cardiomyocytes to provide a novel preclinical approach for the simultaneous prediction of drug-induced inotropic and pro-arrhythmia risks. In order to facilitate the scalability of the model, we recorded fractional sarcomere shortening and then used changes in the contractility transients to infer both inotropic (sarcomere shortening) as well as pro-arrhythmia risk (aftercontraction, AC; contractility escape (CE) and time to 90% relaxation (TR90)). To address the clinical relevance of this approach, we performed a pilot study to test the effects of a large set of reference drugs with well characterized clinical outcomes. Both positive and negative controls were selected, including torsadogenic and non-torsadogenic drugs. We found that the isolated cardiomyocytes exhibited drug-induced contractility changes and pro-arrhythmia markers that are consistent with the known clinical safety profiles of the drugs tested.

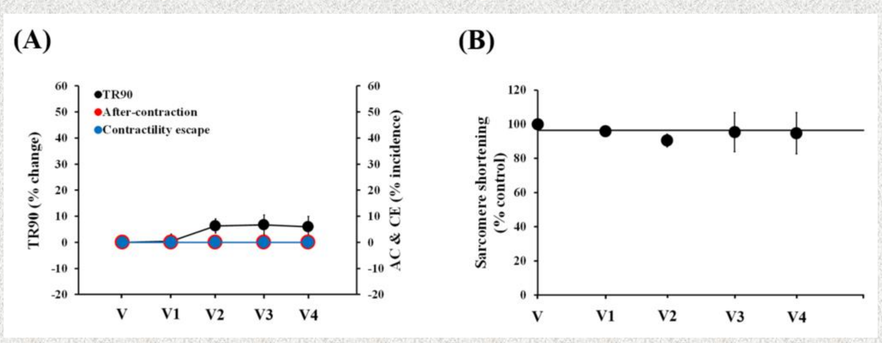
Methods and Selection of drugs

Adult human primary ventricular myocytes isolated from ethically consented donor's hearts were used to measure fractional sarcomere shortening in 1Hz field-stimulation recording using the IonOptix™ system. The stability of sarcomere shortening was assessed by continuous recording for 2 min. in Tyrode's solution establishing the baseline vehicle control (0.1% dimethyl sulfoxide) condition. Test articles were applied for a maximum 250 sec period or when a steady-state effect was achieved. Four ascending concentrations were examined for each test article. Known torsadogenic as well as non-torsadogenic drugs were used in this study. Historical inconsistencies in drug categorization among different studies[1-6] has created some uncertainty in the interpretation of past results. Therefore, we selected molecules from a series of studies where consensus existed with regards to their pro-arrhythmia risk from both the CiPA and the JiCSA initiatives.

Isolated adult human primary ventricular myocytes

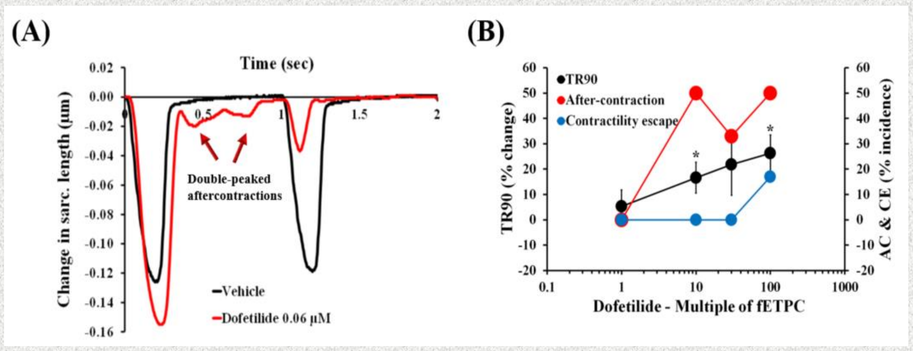
AnaBios has established a novel protocol for the isolation of adult human primary cardiomyocytes. Each isolation yields Ca2+-tolerant cells that retain rod-shaped morphology, exhibit cross striations and contract/relax in response to electrical field stimulation.

Stability of the contractility transients over time



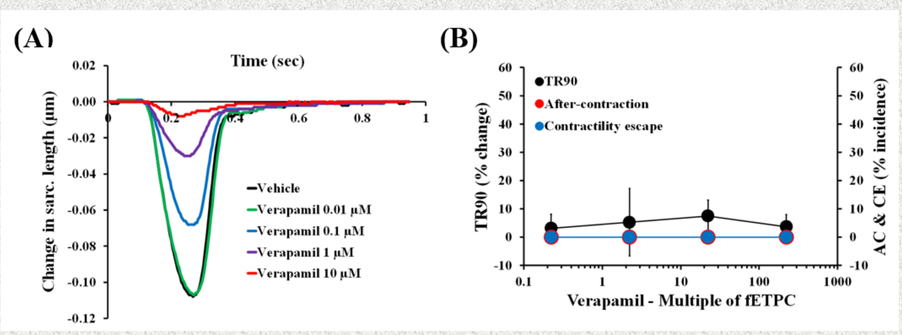
(A) Change in TR90 and % incidence of AC and CE induced by sequential additions of vehicle (V) in human cardiomyocytes at 1Hz pacing frequency. P>0.05 versus V values. **(B)** Vehicle effect curve for sarcomere shortening. V1, V2, V3 and V4 correspond to the 1st, 2nd, 3rd and 4th applications of vehicle.

Human cardiomyocytes predict torsadogenic potential



(A) Typical contractility transients recorded from an adult human primary ventricular myocyte in the presence of vehicle control and after exposure to dofetilide at 0.06 µM (30-fold the fETPC) at a pacing frequency of 1Hz. **(B)** Mean % change in TR90 and AC & CE incidence when cardiomyocytes were incubated with dofetilide at 1Hz. *P<0.05 versus values from vehicle.

Human cardiomyocytes predict safety of verapamil



(A) Typical contractility transients recorded from an adult human primary ventricular myocyte in the presence of vehicle control and after exposure to verapamil at 0.01, 0.1, 1 and 10 µM (0.2-, 2-, 22- and 222-fold the fETPC, respectively) at a pacing frequency of 1Hz. **(B)** Mean % change in TR90 and AC & CE % incidence when cardiomyocytes were incubated with verapamil at 1Hz. P>0.05 versus values from vehicle.

Human cardiomyocyte-based model differentiates between torsadogenic and non-torsadogenic drugs and has excellent sensitivity (95%) and specificity (100%)

Table 1. Pro-arrhythmia prediction of the adult human primary cardiomyocyte-based model

		Pro-arrhythmia risk at 10-fold fETPC					
Drug name	**Clinical TdP risk**	**ANABIOS** Adult human primary ventricular cardiomyocytes (sarcomere shortening)	**AMGEN** hiPSC-derived cardiomyocytes (iCell®, MEA FPD) Qu et al., 2015	**AMGEN** hiPSC-derived cardiomyocytes (iCell®, MEA EAD) Qu et al., 2016	**JiCSA** hiPSC-derived cardiomyocytes (iCell®, MEA Score) Ando et al., 2017	**FDA** hiPSC-derived cardiomyocytes (iCell®, MEA Arrhythmia) Blinova et al., 2017	**FDA** hiPSC-derived cardiomyocytes (Cor.4U, MEA Arrhythmia) Blinova et al., 2017
Ajmaline			Not tested	Not tested		Not tested	Not tested
Astemizole[a]		False negative	Not tested	Not tested		Not tested	Not tested
Azimilide[a]			Not tested	Not tested	Not tested	Not tested	Not tested
Bepridil[a]			Not tested	Not tested	False negative	False negative	False negative
Chlorpromazine[a]			Not tested	Not tested	False negative	False negative	False negative
Cisapride[a]				False negative		False negative	False negative
Clarithromycin[a]			Not tested	Not tested		Not tested	Not tested
Clozapine[a]			Not tested	Not tested	False negative	Not tested	Not tested
D, L-Sotalol[a]						Not tested	Not tested
Disopyramide[a]			Not tested	Not tested		Not tested	Not tested
Dofetilide[a]							
Domperidone[a]			Not tested	Not tested		Not tested	Not tested
Droperidol[a]			Not tested	Not tested		Not tested	Not tested
Erthromycin			Not tested	Not tested		Not tested	Not tested
Flecainide						Not tested	Not tested
Ibutilide[a]			Not tested	Not tested		Not tested	Not tested
Moxifloxacin				Not tested		False negative	False negative
Ondansetron[a]			Not tested	Not tested		Not tested	Not tested
Quinidine[a]			Not tested	Not tested			
Sematilide			Not tested	Not tested		Not tested	Not tested
Terodiline			False negative	False negative		Not tested	Not tested
Vandetanib[a]			Not tested	Not tested		Not tested	Not tested
Diltiazem[a]			Not tested	Not tested			
Diphenhydramine			Not tested	Not tested	False positive	Not tested	Not tested
Loratadine[a]			Not tested	Not tested		Not tested	Not tested
Mexiletine[a]			False positive	Not tested	False positive	Quiescent	
Mibefradil			Not tested	Not tested			
Nifedipine[a]			Not tested	Not tested		Not tested	Not tested
Nitrendipine[a]			Not tested	Not tested		Not tested	Not tested
Ranolazine[a]			False positive		False positive	False positive	
Tamoxifen[a]			Not tested	Not tested		Not tested	Not tested
Verapamil[a]			Not tested	Not tested			Quiescent

[a]: CiPA-selected drug; Red: positive pro-arrhythmia risk; Green: negative pro-arrhythmia risk; hiPSC: human induced pluripotent stem cell (hiPSC); iCell® hiPSC-derived cardiomyocytes from Cellular Dynamics; MEA: micro-electrode array; FPD: Field Potential Duration; JiCSA: Japan iPS Cardiac Safety Assessment; FDA: Food and Drug Administration; Cor.4U: hiPSC-derived cardiomyocytes from Axiogenesis AG; EAD: Early afterdepolarization.

Human cardiomyocytes identify drugs associated with inotropic effects

Table 2. Sarcomere shortening effects for reference drugs measured in adult human primary cardiomyocytes

Drug name	Top test concentration (µM)	Human myocyte effect	IC_{50} (µM)	Ratio (IC_{50}/fETPC)
Ajmaline	1.95	-ve inotrope	2	31
Astemizole[a]	0.009	No effect	>0.009	30
Azimilide[a]	2.1	-ve inotrope	1.07	15
Bepridil[a]	0.96	-ve inotrope	0.7	22
Chlorpromazine[a]	1.04	-ve inotrope	1.02	30
Cisapride[a]	0.26	-ve inotrope	0.02	8
Clarithromycin[a]	120	-ve inotrope	16	13
Clozapine[a]	2.13	-ve inotrope	1.5	21
D, L-Sotalol[a]	450	No effect	>450	>30
Disopyramide[a]	21	-ve inotrope	9.3	13
Dofetilide[a]	0.2	No effect	>0.2	>100
Domperidone[a]	2	-ve inotrope	0.2	10
Droperidol[a]	0.48	-ve inotrope	0.18	11
Erythromycin	5.1	No effect	>5.1	>30
Flecainide	22.6	-ve inotrope	1.1	2
Ibutilide[a]	3	-ve inotrope	2	20
Moxifloxacin	329	No effect	>329	>30
Ondansetron[a]	11.2	-ve inotrope	14	38
Quinidine[a]	100	-ve inotrope	3.6	1
Sematilide	133	No effect	>133	>30
Terodiline	4.35	-ve inotrope	0.7	5
Vandetanib[a]	9	-ve inotrope	2.7	9
Diltiazem[a]	3.84	-ve inotrope	1	8
Diphenhydramine	1.02	-ve inotrope	0.6	18
Loratadine[a]	0.0135	-ve inotrope	0.0175	39
Mexiletine[a]	75	-ve inotrope	0.9	0.4
Mibefradil	0.36	-ve inotrope	0.18	15
Nifedipine[a]	0.23	-ve inotrope	0.04	5
Nitrendipine[a]	0.091	-ve inotrope	0.06	20
Ranolazine[a]	200	-ve inotrope	17	9
Tamoxifen[a]	0.663	-ve inotrope	0.99	45
Verapamil[a]	10	-ve inotrope	0.09	2

IC_{50}; Concentration inducing 50% decrease in sarcomere shortening; Hill equation using SigmaPlot v13 was fitted to sarcomere shortening concentration-effect curves, assuming drugs would eventually cause complete inhibition of the contractility when they decreased sarcomere shortening by ≥25%. [a]: CiPA-selected drug; fETPC, free effective therapeutic plasma concentration.

Summary

1. Adult human primary cardiomyocytes can simultaneously predict risks associated with pro-arrhythmia and inotropic activity.
2. The human primary cardiomyocyte model enables the generation of reliable and predictive data for human-focused cardiac safety assessment at early stages in drug discovery.
3. The adult human primary cardiomyocyte model appears to be more predictive of drug-induced cardiotoxicity than the stem cell-derived cardiomyocyte models.

REFERENCES

[1]Colatsky T et al., JPTM 81 (2016) 15-20; [2]CredibleMeds, www.crediblemeds.com; [3]Haverkamp W et al., EHJ 3 (Supplement K) (2001) K81-K88; [4]Redfern WS et al., Cardiovasc Res 58(1) (2003) 32-45; [5]Poluzzi E et al., Pharmacoepidemiol Drug Saf 18(6) (2009) 512-518; [6]Blinova K et al., Toxicol Sci 155(1) (2017) 234-247; [7]Qu Y & Vargas H, Toxicol Sci 147(1) (2015) 286-295; [8]Ando H et al., JPTM 84 (2017) 111-127