Adult human primary cardiomyocytes: an integrative translational model for preclinical drug testing

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Abstract

Human adult cardiac tissue provides a much-needed integrative preclinical model to reliably assess the toxicity risks of new drugs. To this aim, we have established methodologies that consistently allow the procurement and experimental interrogation of human heart tissue preparations. These ex-vivo cardiac models enable drug discovery projects to generate predictive human-based data at the preclinical stage. In order to grow the throughput and scalability of the human ex-vivo heart model, we have developed novel methodologies for the isolation of adult human primary cardiomyocytes. Each isolation yields Ca^{2+}-tolerant cells that retain rod-shaped morphology, exhibit cross striations and contract/relax in response to field electrical stimulation. The cells also display the ability to adapt to changes in cycle length. To validate the use of these cells in predicting drug effects, we have assessed the effects of reference drugs on the excitation-contraction coupling. Specifically, we have measured the effects of reference drugs with known degrees of risk in human, similarly to the validation strategy adopted by the Comprehensive In Vitro Pro-arrhythmia Assay (CiPA) initiative. The validation of human primary myocytes for the assessment of drug risk potential will provide an important preclinical tool for risk assessment. In addition to the study of normal adult myocytes described in the present abstract, the opportunity now exists for the use of adult cardiomyocytes from highly prevalent disease conditions (diabetes, cardiac hypertrophy, heart failure, etc.), and therefore, for the ability to assess how cardiac toxicity risk may be affected by common comorbidities.

Isolation of primary cardiomyocytes from human hearts

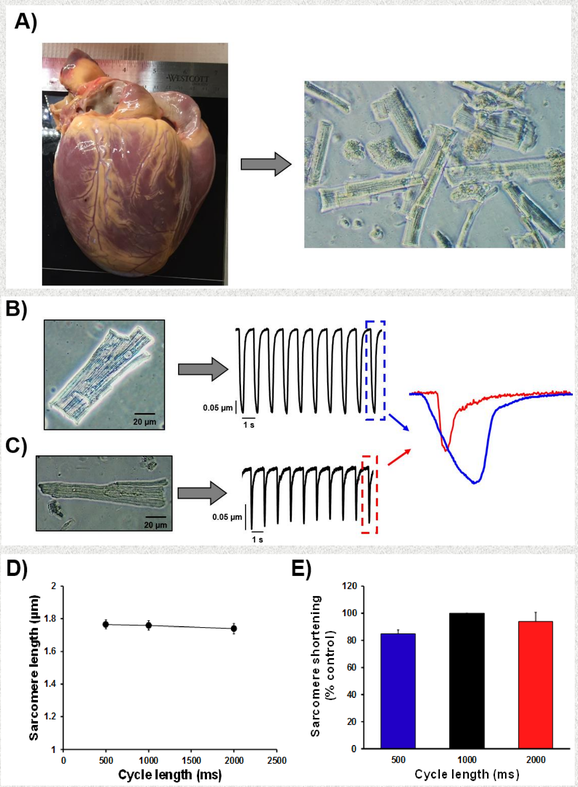

Figure 1A. Shows a typical human heart that AnaBios uses to isolate human myocytes.
Isolated cardiomyocytes were found to be Ca^{2+}-tolerant, retain rod-shaped morphology and exhibit cross striations.

Figure 1B and C. Phase contrast microscopy images of representative human primary ventricular (**1B** left) and atrial (**1C** left) myocytes. Typical contractility transients recorded from these two cardiomyocytes at a cycle length of 1000ms are also shown.

Figure 1D and E. (**D**) shows the average sarcomere length for human cardiomyocytes in vehicle conditions at different cycle lengths. (**E**) Rate adaptation was assessed by measuring sarcomere shortening in vehicle conditions with changing cycle length from long to short cycles. Effects were expressed as the % control compared to the myocyte's specific baseline 1000ms period. Results are expressed as mean ± SEM. n=3.

Human primary ventricular myocyte-based model predicts torsadogenic potential of quinidine

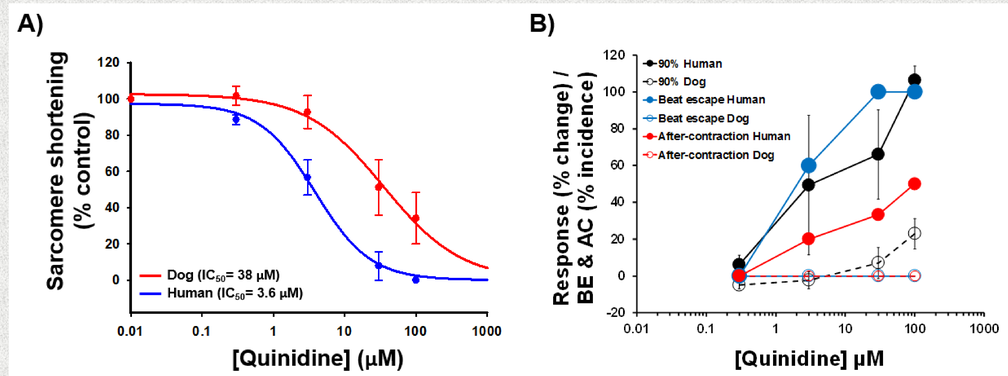

Figure 3. (**A**) IC_{50} for the sarcomere shortening-inhibitory effect of quinidine was 11-fold more potent in human ventricular myocytes (n=5) compared to dog cells (n=5) at 1000ms cycle length. IC_{50}: concentration of the drug producing 50% inhibition in sarcomere shortening. The 0.01 µM represents the normalized vehicle data. (**B**) Effects of quinidine on time to 90% relaxation, a parameter of the contractility transient. Quinidine elicited a significantly higher increase in this parameter in myocytes from human hearts compared to dog hearts. Similar findings were observed for times to 30% and 50% relaxation (data not shown). BEs and ACs were only seen in quinidine-treated human myocytes. Treatment effects were expressed relatively to the myocyte's specific baseline control period. Results are expressed as mean ± SEM.

Human primary ventricular myocyte-based model predicts safety of verapamil

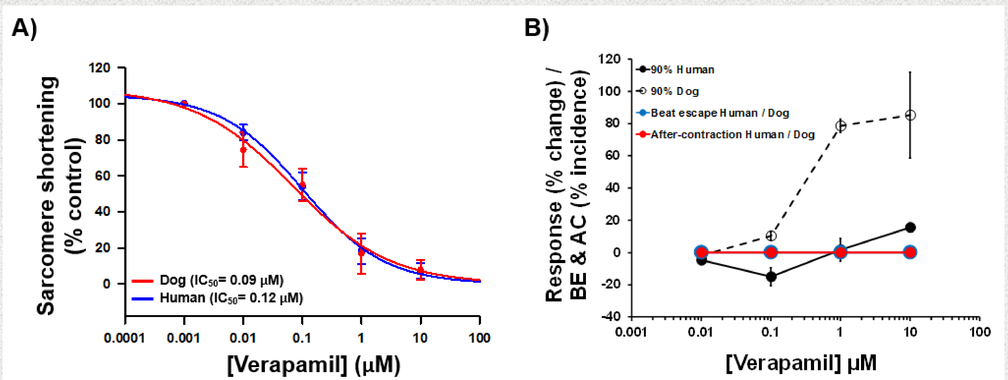

Figure 4. (**A**) Verapamil's potency was similar for the human ventricular myocytes (n=4) to that obtained in dog cells (n=4) at 1000ms cycle length. The 0.001 µM represents the normalized vehicle data. (**B**) Effects of verapamil on time to 90% relaxation. Verapamil elicited a significantly higher increase in this parameter in myocytes from dog compared to human hearts. Similar findings were observed for times to 30% and 50% relaxation (data not shown). No BEs or ACs were seen in any verapamil-treated human or dog myocytes. Treatment effects were expressed relatively to the myocyte's specific baseline control period. Results are expressed as mean ± SEM.

Materials and Methods

We used single adult human primary ventricular myocytes isolated from ethically consented donor's hearts to measure fractional sarcomere shortening in field-stimulation recording using a digital, cell geometry measurement system (IonOptix™). A comparative set of experiments were also performed on ventricular myocytes isolated from beagle dog hearts as previously described[1]. Sarcomere shortening stability was assessed by continuous recording for 1-2min in Tyrode's solution establishing our control vehicle (0.1% dimethyl sulfoxide) condition. Then, the test item concentration was applied for a minimum of 250sec period or when a steady-state effect was achieved sometime at a smaller exposure period. Four ascending concentrations of the test items were tested allowing cumulative concentration-effect curves to be determined. Test items consisted of a pro-arrhythmic (quinidine) or non-pro-arrhythmic (verapamil) drug.

Effects of quinidine on human and dog cardiomyocyte contractility

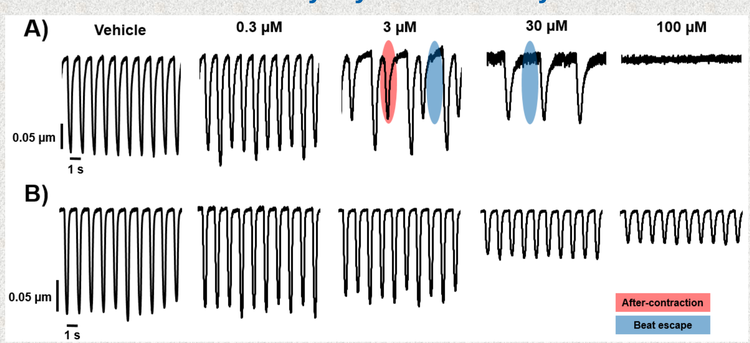

Figure 2. Typical contractility transients recorded from human (**A**) and dog (**B**) cardiomyocytes exposed to the vehicle and four ascending concentrations of quinidine at a cycle length of 1000ms. Beat escape (BE): electrical stimulus did not trigger a contraction following relaxation. After-contraction (AC): an abnormal and unsynchronized contraction. Transients are shown for a 10-sec period.

Summary

1. Using the novel isolation protocol we have developed, the human primary ventricular myocyte-based model reproduces established electrophysiological characteristics of cardiomyocytes.
2. Our model provides the opportunity to clearly differentiate between quinidine (a pro-arrhythmic drug) and verapamil (a non-pro-arrhythmic drug).
3. A larger validation is underway to fully evaluate the performance of this ex-vivo model.
4. Our model has the potential to enable, for the first time, the generation of human-based cardiotoxicity data at the preclinical stage.

[1]Abi-Gerges N et al., JMCC 64 (2013) 108-119