A human ex-vivo contractility-based assay for the simultaneous prediction of drug-induced inotropic and pro-arrhythmia risk

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Abstract

Cardiac safety remains the leading cause of drug development discontinuation and withdrawal of post marketing approvals. This has called into question the reliability of current preclinical safety-testing paradigms, which rely predominantly on animal models, and has led to demands for more predictive tools. To this aim, we sought to develop and validate a new ex-vivo human-based model that uses human ventricular trabeculae, paced under tension and combined with continuous recordings of the exerted force of contraction (Fc). We were especially interested in establishing if this strategy could provide a more predictive approach for assessing both inotropic activity as well as pro-arrhythmia risk. Since the T-wave recorded in ECG in the clinics marks ventricular repolarization and is therefore an electrical measure of the contraction's relaxation, we used Fc parameters to assess drug-induced inotropic effect (maximum amplitude of contraction; MAC), pro-arrhythmia (after-contraction, AC which has been proposed to be triggered by early-afterdepolarizations) and QT prolongation (TR70, time to 70% relaxation). We generated an initial set of validation data employing reference drugs that included isoproterenol (sympathomimetic), verapamil (non-torsadogenic), dofetilide and d,l-sotalol (both torsadogenic). Each drug was tested separately in 5 ascending concentrations. We found that dofetilide and sotalol had no effects on MAC, while isoproterenol increased MAC (by 315% at 0.03µM) and verapamil decreased it (IC_{50}=1.46µM). Dofetilide and sotalol both induced ACs starting at the free Effective Therapeutic Plasma Concentration (fETPC), while verapamil and isoproterenol did not induce any ACs even when tested up to 222x and 1000x of their fETPCs, respectively. Finally, concentration-dependent increase in TR70 was seen with dofetilide and sotalol, while isoproterenol induced a decrease in TR70. Verapamil had no effect on TR70 up to 222x the fETPC. In summary, these data demonstrate that the human ex-vivo tissue-based model has the potential to simultaneously predict risk associated with inotropic activity and QT/pro-arrhythmia.

Materials and Methods

Ventricular trabeculae were dissected from 4 ethically consented donor's hearts[1]. Each trabecula was mounted in a vertical double-jacketed organ bath equipped with a force transducer and containing oxygenated Tyrode's solution warmed to 36°C. Trabeculae were then equilibrated, while applying tension. Contraction was initiated by applying a fixed 1Hz stimulation. Recordings were performed in continuous mode with 10KHz sampling using AD Instruments and LabChart Software. Each drug concentration was applied for 20 min and the last 60 contractions acquired at the end of the 20-min period were averaged. Treatment effects were expressed relatively to each trabecula specific baseline measurements. Results are expressed as mean ± S.E.M.

Human ventricular trabeculae-based model predicts the torsadogenic potential of dofetilide and d,l-sotalol

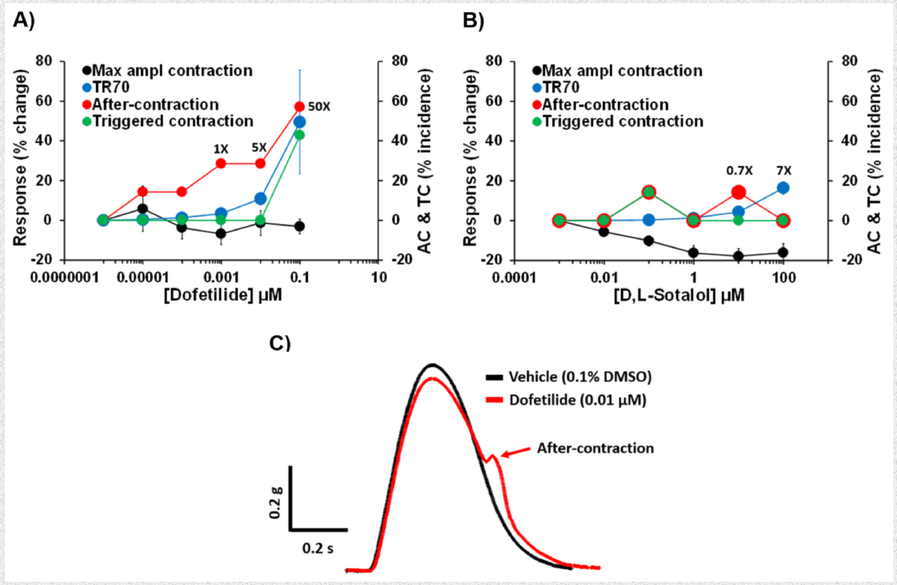

Figure 1. Effects of dofetilide (**A**) and d,l-sotalol (**B**) on Fc parameters in human ex-vivo ventricular trabeculae. Neither dofetilide nor d,l-sotalol affected the maximum amplitude of contraction. However, in the same tissue samples, dofetilide and d,l-sotalol increased TR70 and induced ACs and triggered contractions. AC incidence with both drugs was seen starting at the fETPC. n=7 trabeculae for each drug. Note that dofetilide and d,l-sotalol are not associated with clinical inotropic risk. (**C**) shows example of an AC elicited in the presence of dofetilide at 10-fold the fETPC.

Dofetilide induced after-contractions and triggered contractions in human ventricular trabeculae

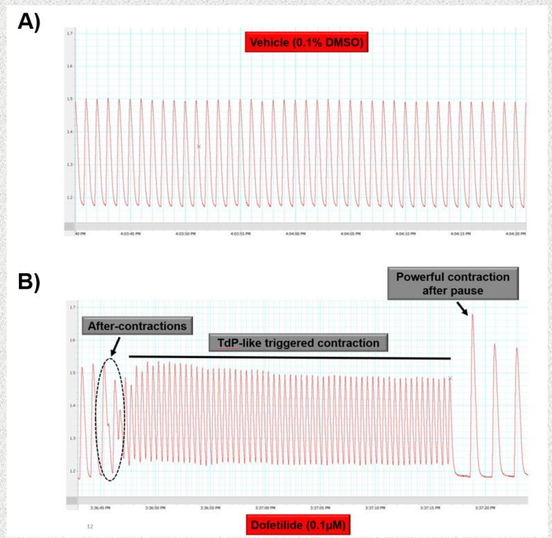

Figure 2. (**A**) Example of normal contraction/relaxation cycles elicited in the presence of vehicle. (**B**) Example of dofetilide-induced ACs and a torsadogenic-like triggered contraction at 50-fold the fETPC. The torsadogenic-like triggered contraction lasted for approx. 1-min and was self-terminated. It was followed by a pause with a subsequent beat showing marked contraction and subsequently, normal contraction/relaxation cycles resumed. Note that TdP (Torsades de Pointes) arrhythmia has been proposed to arise from premature ventricular contractions (i.e., AC events in our study) due to triggered early after-depolarizations.

Human ventricular trabeculae-based model predicts safety of verapamil

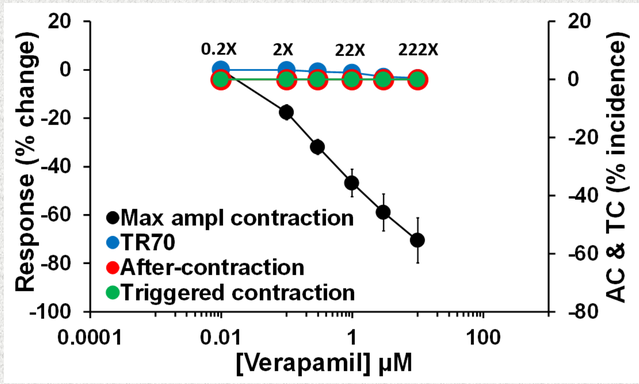

Figure 3. Effects of verapamil on Fc parameters in human ex-vivo ventricular trabeculae. Verapamil decreased the force of contraction with an IC_{50} value of 1.46µM, while it had no effect on TR70 and did not induce any ACs or triggered contractions when tested up to 222-fold its fETPC. n=7 trabeculae. Note that the negative inotropic effect of verapamil is in line with its known inhibition of Ca^{2+} channel).

Human ventricular trabeculae-based model predicts effects of isoproterenol on force of contraction

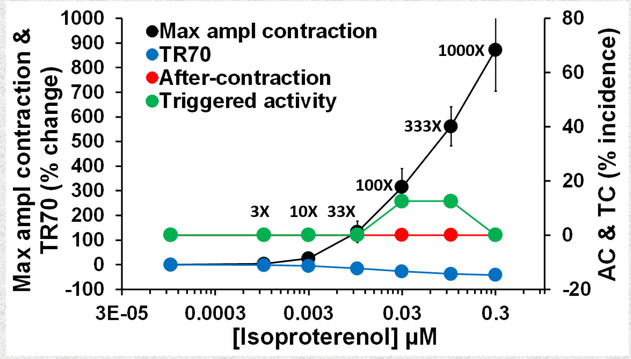

Figure 4. Effects of isoproterenol on Fc parameters in human ex-vivo ventricular trabeculae. Isoproterenol increased the force of contraction (for example by 315% at 0.03µM). While isoproterenol induced a decrease in TR70 (i.e., lusitropic effect, it did not induce any ACs when tested up to 1000-fold its physiological level. Incidence of triggered contraction was seen only in 1 out of 8 trabeculae at 100- and 333-fold the physiological level. n=8 trabeculae.

Summary

1. The human ventricular trabeculae-based model can clearly differentiate between pro-arrhythmic and non-proarrhythmic drugs.
2. This model has also the potential to simultaneously predict risk associated with inotropic activity and QT/pro-arrhythmia.
3. The human ventricular trabeculae-based model has the potential to enable, for the first time, the generation of reliable and predictive human-based cardiotoxicity data at the preclinical stage.

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