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

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Abstract

Cardio 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 (IC50=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 22X and 100X 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. 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 23-min period were averaged. Treatment effects were expressed relatively to each trabecula specific baseline measurements. Results are expressed as mean ± S.E.M.

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.