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Research article

Significance of integrated *in silico* transmural ventricular wedge preparation models of human non-failing and failing hearts for safety evaluation of drug candidates

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ABSTRACT

Introduction: To evaluate the usefulness of *in silico* assay in predicting drug-induced QTc prolongation and ventricular proarrhythmia, we describe in this study 2-dimensional transmural ventricular wedge preparation model (2D model) of non-failing (non-FH) and failing hearts (FH) based on O'Hara-Rudy dynamic model of human ventricular myocytes.

Methods: Using the prepared 2D model, we simulated ventricular action potential and recorded electrocardiogram for the non-FH and FH. The FH model was constructed based on differences in mRNA, protein, and/or current levels of ion channels between non-diseased heart and failing heart. To simulate the effects of selected drugs, we incorporated changes in ion channel conductance depending on the IC_{50} value and Hill coefficient at unbound drug blood concentrations.

Results: Dofetilide concentration-dependently induced QTc prolongation at therapeutic concentration in the 2D model of both non-FH and FH. The QTc prolongation in FH was longer than that in non-FH. These findings are consistent with previously reported clinical data. At supratherapeutic concentration 20 nM, dofetilide induced Torsade de Pointes-like arrhythmia in the 2D non-FH model. In contrast, the single ventricular myocyte model did not quantitatively reproduce experimental data due to lack of electrotonic interaction. The simulated QTc change induced by six drugs examined in the IQ-CSRC prospective study was almost equivalent to that recorded in drug-treated healthy volunteers.

Discussion: Our 2D model with or without heart failure faithfully reproduced drug-induced QT prolongation and ventricular arrhythmias, suggesting that the *in silico* approach is a powerful tool for predicting cardiac safety of drug candidates at preclinical stage.

1. Introduction

Current approaches for evaluation of preclinical drug candidates' potential for delayed ventricular repolarization are based on ICH, S7B guidelines, which require performance of both hERG current assay and *in vivo* QT assay for drugs in preclinical stage and QTc prolongation test for drugs in clinical development. However, it has recently been suggested that such approaches are inadequate for predicting drug candidates' potential for proarrhythmia. Accordingly, the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) has been proposed as a novel strategy intended to replace the guidelines with a multi-channel assay, *in silico* assay, and a myocyte assay that uses human induced pluripotent stem cell-derived cardiomyocytes (Cavero & Holzgrefe, 2014; Colatsky et al., 2016).

Among these assays, the *in silico* assay of human ventricular action potential (AP) is expected to be one of the most useful tools for

Abbreviations: AP, action potential; APD, action potential duration; APD_{30}, APD at 30% repolarization; APD_{90}, APD at 90% repolarization; APD_{30-90}, APD_{90}–APD_{30}; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; CiPA, Comprehensive *in vitro* Proarrhythmia Assay; CV, conduction velocity; EAD, early afterdepolarization; ECG, electrocardiogram; Endo, endocardial; Epi, epicardial; FH, failing heart; ICH, International Conference of Harmonization; IQ-CSRC, Innovation and Quality in Pharmaceutical Development-Cardiac Safety Research Consortium; I_{CaL}, L-type Ca^{2+} current; I_{Kr}, rapid delayed rectifier potassium current; I_{Ks}, slow delayed rectifier potassium current; I_{K1}, inward rectifier potassium current; I_{NaCa}, Na^+/Ca^{2+} exchanger current; I_{NaF}, fast component of Na^+ current; I_{NaK}, Na^+/K^+ pump current; I_{NaL}, late component of Na^+ current; I_{rel}, SR Ca^{2+} release flux *via* Ryanodine receptor; I_{to}, transient outward potassium current; I_{up}, Ca^{2+} uptake *via* SERCA pump; M, midmyocardial; ORd, O'Hara-Rudy dynamic; PCL, pacing cycle length; QTc, corrected QT interval; TdP, Torsade de Pointes; Tpe, T peak to end; tT, ten Tusscher; TQT study, Thorough QT study; VM, ventricular myocyte; 0D, cellular level; 1D, 1-dimensional; 2D, 2-dimensional; 2D model, 2D transmural ventricular wedge preparation model; 3D, 3-dimensional.

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characterizing drug candidates' electrophysiological effects on multiple human cardiac currents. Indeed, the O'Hara-Rudy dynamic (ORd) human ventricular AP model, which was constructed based on data from over 100 non-diseased human ventricles, is currently believed to be the most suitable model for simulations of ventricular proarrhythmia (O'Hara et al., 2011; Pugsley et al., 2014). Mirams et al. (2014) also reported that AP simulation can be a useful tool for predicting drugs cardiac safety and provided a web portal for *in silico* AP prediction using various human and animal ventricular AP models (Williams & Mirams, 2015). On the other hand, it has been reported that there are measurable differences in AP upstrokes between simulation of a single ventricular myocyte (VM) at the cellular level (0D) and simulation at tissue level (Elshrif, Pengcheng, & Cherry, 2014), with tissue level simulation being a more useful approach for predicting drug safety in the human heart. Indeed, several tissue simulation studies aimed at predicting drug effects on ECG have recently been reported. Polak et al., for instance, evaluated QTc changes following administration of antipsychotic drugs and Quinidine using a transmural 1-dimensional (1D) model based on ten Tusscher (tT) and ORd membrane kinetics (Glinka & Polak, 2014; Polak, 2013). Moreno et al. (2011), on the other hand, predicted drug effects on reentry induction in 1D and 3-dimensional (3D) ventricular models based on tT membrane kinetics, and indicated differences in reentry inducibility between the failing heart (FH) and non-FH. Okada et al. (2015) also numerically evaluated drug-induced arrhythmia using an integrated 3D ventricular model. The usefulness of ECG simulation for evaluation of drug safety will be probably discussed in future reports.

Current approaches for evaluation of drug safety focus only on non-diseased state, because it is still very difficult to predict drug effects in diseased state using conventional pharmacological tests. *In silico* assay would be the only ethical technique that can help predict drug safety. Using differences in ion channel function between FH and non-FH, Elshrif et al. (2014) reported new AP models of FH based on ORd and Priebe-Beuckelmann human ventricular models (Priebe & Beuckelmann, 1998). In another study using transmural FH 1D model, Gomez et al. (2014) reported that both increase in electrophysiological gradient and reduction in electrical propagation result in proarrhythmia. However, no report has so far compared simulated ECG and clinical ECG in patients with FH. In addition, no report has simulated drug effects on ECG in patients with FH.

Previously, we reported the preparation of a 2D transmural ventricular wedge model (2D model) of non-FH and FH based on the ORd model (Kubo et al., 2015). Here, we evaluated the usefulness of this model in predicting drug-induced proarrhythmia in preclinical studies and show possible mechanism of Torsade de Pointes (TdP)-like arrhythmia induced by dofetilide. Dofetilide, a major I_{Kr} blocker, was used as positive control. To validate the 2D model, we evaluated selected drugs effects on QTc in simulated human ECG and compared the results to clinical data reported in the IQ-CSRC prospective study. The IQ-CSRC prospective study as performed by Darpo et al. (2015), is considered as an appropriately designed, well executed study equivalent to a formal Thorough QT (TQT) study.

2. Methods

2.1. Computer simulation

Based on the ORd model, we simulated selected drug effects on AP and ECG in the 0D model (single myocyte) and in the bidomain 2D wedge preparation model (4.5 cm in length and 1.01 cm in thickness). The 2D non-FH model was paced from a section of the endocardial border (1.1 cm in length), thereby mimicking transmural propagation during sinus rhythm within the ventricular free wall. To obtain ventricular transmural gradient, endocardial (Endo), midmyocardial (M), and epicardial (Epi) layers with a thickness of 0.12 cm, 0.65 cm, and 0.24 cm, respectively (Fig. 1A) were selected. As modification of the ORd model is also required to construct a tissue model (O'Hara et al., 2011), I_{NaF} was amplified to adjust conduction velocity (CV) of the 2D model to clinical values (Taggart et al., 2000). A 2-ms pacing stimuli with a strength twice the diastolic threshold was trans-membranously applied to the endocardial end at a pacing cycle length (PCL) of 500, 1000, 2000, and 4000 ms. To obtain an ECG similar to that of the left precordial ECG, a unipolar recording electrode was applied 2.6 cm above the epicardial end of the tissue. To achieve transmural difference in CV, fiber orientation of the vertical cross-section of 3D rotational anisotropy was incorporated in the system (Ashihara et al., 2003). Other model parameters, such as tissue conductivity and boundary conditions, were selected as described elsewhere (Ashihara & Trayanova, 2004). We also simulated excitation propagation in tissue using imaging of membrane potential. To construct the FH model, we first referred to the data provided in the original ORd model, because the non-FH model used in this study is mainly based on the ORd model. O'Hara et al. (2011) selected reliable literature to reproduce human ventricular AP, and the ORd model is currently believed to be the most suitable model for simulation of ventricular proarrhythmia. We referred the work published by Soltysinska et al. (2009), which includes a comparison of mRNA level between non-FH and FH. With this bottom-up approach, we added a "top-down" approach based on clinical ECG data for FH to reproduce a FH condition (Khan et al., 2007). Finally, we considered published data on current density and membrane capacitance to fit clinical papers (Beuckelmann et al, 1993; Konarzewska et al., 1995; Nabauer et al, 1996; Schwinger et al, 1999; Valdivia et al., 2005), because some of the parameters could not be determined from mRNA change. For example, I_{NaF} and I_{NaL} current amplitudes could not be determined from mRNA level. Current amplitude, protein expression and m-RNA level for all ion channels, exchangers, and VM capacitance of FH patient (Fig. 2) as well as differences in all parameters between non-FH and FH are shown in Supplementary Table 1. Computation was performed on a linux workstation and a laptop computer using a program coded by the "C" language.

2.2. Simulation experimental and clinical data

To simulate the effects of selected drugs, each ion channel conductance was changed depending on the IC_{50} value and Hill coefficient (nH) at unbound blood concentration. Dofetilide IC_{50} value for I_{Kr} (9.76 nM) and Hill coefficient (0.89) by conventional patch clamp system at physiological temperature were obtained from the literature (Du, El Harchi, Zhang, Orchard, & Hancox, 2011). Dofetilide protein binding rate (64%) and clinical ECG data were obtained from the literature (Pfizer Labs, 1999; Sedgwick, Rasmussen, Walker, & Cobbe, 1991). Experimental data for simulated drugs, Dofetilide (CID: 71329), Hydrodolasetron (CID: 52950077), Levocetirizine (CID: 1549000), Moxifloxacin (CID: 152946), Ondansetron (CID: 4595), and Quinine (CID: 3034034), were shown in the Table 1. Clinical data of QTc change were those published in the IQ-CSRC prospective study (Darpo et al., 2015).

2.3. Simulation condition and data analysis

The effects of dofetilide on AP were simulated at 5, 10, 15, 20, 25, 30, 40, 50, 100, 200 and 300 nM in the 0D model. The shape and duration of the simulated AP were determined as early afterdepolarization (EAD), AP duration (APD) at 90% repolarization (APD_{90}), and APD_{90} minus APD at 30% repolarization (APD_{30-90}). The effects of dofetilide on ECG were simulated at 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 ng/mL to predict QT prolongation, and at 5, 10, 15, 20, 25, and 30 nM to evaluate threshold concentrations for proarrhythmia. The effects of six drugs reported in the IQ-CSRC prospective study on QTc were simulated at wide range of plasma concentrations including supratherapeutic concentration. QRS duration, QT interval, and Tpeak-to-end (Tpe) were measured based on the simulated ECG. QT interval was determined by Fridericia's formula ($QTc = QT / RR^{1/3}$).