Journal of Pharmacological and Toxicological Methods 87 (2017) 68–73

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Brief communication

Computational modeling for cardiac safety pharmacology analysis: Contribution of fibroblasts

Xin Gao[a], Tyler Engel[b], Brian E. Carlson[a], Tetsuro Wakatsuki[b,*]

[a] Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI, United States
[b] InvivoSciences, Inc., Madison, WI, United States

A B S T R A C T

Introduction: Drug-induced proarrhythmic potential is an important regulatory criterion in safety pharmacology. The application of *in silico* approaches to predict proarrhythmic potential of new compounds is under consideration as part of future guidelines. Current approaches simulate the electrophysiology of a single human adult ventricular cardiomyocyte. However, drug-induced proarrhythmic potential can be different when cardiomyocytes are surrounded by non-muscle cells. Incorporating fibroblasts in models of myocardium is important particularly for predicting a drugs cardiac liability in the aging population – a growing population who take more medications and exhibit increased cardiac fibrosis. In this study, we used computational models to investigate the effects of fibroblast coupling on the electrophysiology and response to drugs of cardiomyocytes.

Methods: A computational model of cardiomyocyte electrophysiology and ion handling (O'Hara, Virag, Varro, & Rudy, 2011) is coupled to a passive model of fibroblast electrophysiology to test the effects of three compounds that block cardiomyocyte ion channels. Results are compared to model results without fibroblast coupling to see how fibroblasts affect cardiomyocyte action potential duration at 90% repolarization (APD_{90}) and propensity for early after depolarization (EAD).

Results: Simulation results show changes in cardiomyocyte APD_{90} with increasing concentration of three drugs that affect cardiac function (dofetilide, vardenafil and nebivolol) when no fibroblasts are coupled to the cardiomyocyte. Coupling fibroblasts to cardiomyocytes markedly shortens APD_{90}. Moreover, increasing the number of fibroblasts can augment the shortening effect.

Discussion: Coupling cardiomyocytes and fibroblasts are predicted to decrease proarrhythmic susceptibility under dofetilide, vardenafil and nebivolol block. However, this result is sensitive to parameters which define the electrophysiological function of the fibroblast. Fibroblast membrane capacitance and conductance (C_{FB} and G_{FB}) have less of an effect on APD_{90} than the fibroblast resting membrane potential (E_{FB}). This study suggests that in both theoretical models and experimental tissue constructs that represent cardiac tissue, both cardiomyocytes and non-muscle cells should be considered when testing cardiac pharmacological agents.

1. Introduction

Cardiomyocytes occupy a major part of the heart muscle by volume, yet > 65% of cells in myocardium are non-muscle cells (Bergmann et al., 2015; Nag, 1980; Pinto et al., 2016). *In vitro* experiments have been used to confirm significant effects of the coupling between cardiomyocytes and non-muscle cells on cardiomyocyte electrophysiology (Kohl & Gourdie, 2014). While the critical role of electrical coupling between cells *in vivo* is still under discussions (Kohl & Gourdie, 2014), myocyte-fibroblast coupling has been recorded in healthy hearts and more clearly in those in cardiac remodeling after injury or chronic stress (Ongstad & Kohl, 2016).

The aging population (> 65 years of age) is the largest group of consumers of pharmaceuticals many of which are taken to treat a high prevalence of chronic heart failure and fibrillation (Vigen, Maddox, & Allen, 2012). One distinct feature of the aging heart is its increased number of non-muscle cells including myofibroblasts that contribute to fibrosis (Biernacka & Frangogiannis, 2011). This large aging population is expected to grow in accordance with the increasing average life expectancy of the world population and pharmaceuticals targeted to this group must be screened for the possibility of adverse cardiac effects accounting for the contribution of non-muscle cells to

* Corresponding author.
E-mail address: tetsuro@invivosciences.com (T. Wakatsuki).

http://dx.doi.org/10.1016/j.vascn.2017.04.011
Received 17 February 2017; Received in revised form 15 April 2017; Accepted 25 April 2017
Available online 26 April 2017
1056-8719/

cardiomyocyte electrophysiology.

One of the primary objectives of the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) is to use *in silico* simulations based on the O'Hara-Rudy (ORd) model (O'Hara, Virag, Varro, & Rudy, 2011) for proarrhythmic potential of compounds exhibiting inhibition of one or more cardiomyocyte ion channels (*e.g.*, rapid delayed rectifier K^+, L-type Ca^{2+} and/or fast Na^+ channels). When using the ORd model to predict cardiomyocyte electrophysiological function in cardiac tissue an understanding of the limitations and scope of the model is important. Like conducting any bench-top experiments, the use of theoretical models must match the question asked. Here, we introduce a simple extension of the ORd model to simulate electrophysiology of an adult cardiomyocyte connected to cardiac fibroblasts and how this computational model responds to the application of cardiac sensitive drugs. The ORd model was chosen as the foundational model to use here because it was developed based on experimental data conducted using human adult ventricular cardiomyocytes isolated from healthy donors and has been adopted as a starting point for the CiPA project aimed at supporting regulatory decisions with respect to cardiac drug proarrhythmic risk (Sager, Gintant, Turner, Pettit, & Stockbridge, 2014).

Multiple experimental analyses using *in vitro* models (Vasquez, Benamer, & Morley, 2011; Xie et al. 2009) and also computational analyses (Nayak, Shajahan, Panfilov, & Pandit, 2013; Sridhar, Vandersickel, & Panfilov, 2017) support the idea that cardiac fibroblasts affect the arrhythmogenicity of the myocardium including changes in action potential duration and early after depolarization (EAD). However, how the connection between fibroblasts and cardiomyocytes and the current flow through that connection can change the overall electrophysiology of myocardium has not been explored in the context of cardiac sensitive drug response. Specifically, the effects of the magnitude of the gap junctional conductance and the underlying electrophysiology of cardiac fibroblasts has not yet been evaluated quantitatively in the analysis of proarrhythmia risk.

The coupled model presented here, developed in response to these issues, simulates drug-induced changes in electrophysiology not solely in isolated cardiomyocytes but in cardiac tissue which is comprised of both cardiomyocytes and non-muscle cells. In this study, we aim to show how a simple modification of the ORd model can influence the predicted susceptibility of cardiac tissue to the effects of proarrhythmic compounds.

2. Methods

Electrical coupling between cardiomyocytes and fibroblasts were modeled by assuming that a cardiomyocyte is surrounded by N fibroblasts, and the gap junction between cardiomyocyte and fibroblast is an electrical conductor (Fig. 1). The fibroblast itself was modeled as an electrically passive cell (Kohl, Kamkin, Kiseleva, & Noble, 1994). Thus, the membrane potential of the cardiomyocyte (V_{CM}) and fibroblast (V_{FB}) can be written as:

$$C_{CM}\frac{dV_{CM}}{dt} = -\left(I_{CM} + \sum_{k=0}^{N} I_{gap}^{k} + I_{stim}\right), \quad (1)$$

$$C_{FB}\frac{dV_{FB}}{dt} = -(I_{FB} + I_{gap}), \quad (2)$$

$$I_{FB} = G_{FB}(V_{FB} - E_{FB}), \quad (3)$$

$$I_{gap} = G_{gap}(V_{CM} - V_{FB}), \quad (4)$$

where C_{CM} and C_{FB} are the membrane capacitances of the cardiomyocyte and fibroblast, I_{CM} and I_{FB} are the membrane currents of the cardiomyocyte and fibroblast, I_{stim} is the stimulus current, G_{FB} and E_{FB} are the electrical conductance and resting potential of the fibroblast, and I_{gap} and G_{gap} are the current and electrical conductance of gap junction.

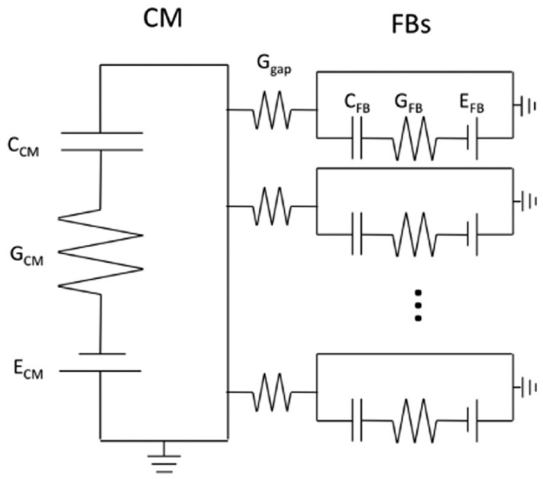

Fig. 1. Schematic of cardiomyocyte coupled with one or more fibroblasts. Conductance across the cell membrane in the fibroblast, G_{FB}, is fixed while the conductance across the cardiomyocyte membrane, G_{CM}, is governed by the dynamics of the ORd model. Gap junction conductance (G_{gap}), fibroblast conductance (G_{FB}), fibroblast membrane capacitance (C_{FB}) and fibroblast membrane Nernst potential (E_{FB}) are chosen from studies by Kohl et al. (Kohl et al., 1994) and Xie et al. (Xie, Garfinkel, et al., 2009). Cardiomyocyte membrane capacitance is not explicitly used in the ORd model so the value (185 pF) used in ten Tusscher et al. (ten Tusscher et al., 2004) was used when fibroblasts were coupled to the cardiomyocyte model. Ratio of the number of fibroblast cells to cardiomyocyte cells is typically believed to be between 2 and 3 in normal tissue (Rohr, 2012).

The cardiomyocyte membrane potential and current were simulated with the ORd model which is capable of reproducing experimentally observed human adult ventricular cardiomyocyte action potential shapes with and without the blocking of specific ion channels (O'Hara et al., 2011). The fibroblast membrane conductance (G_{FB}) was chosen as 0.5 nS which is in the range of experimental results (0.1 to 4 nS) (Kohl et al., 1994). The conductance of gap junction (G_{gap}) was chosen as 1 nS (Kohl et al., 1994). The membrane capacitance of the fibroblast (C_{FB}) was chosen as 25 pF, and the resting potential (E_{FB}) was chosen as − 50 mV (Xie, Garfinkel, Weiss, & Qu, 2009).

The blocking of cardiomyocyte membrane ion channel currents by compounds were modeled by reducing the maximal ion channel conductance as a function of compound concentration. A simple relationship between the ion channel conductance and the compound concentration is given by the Hill equation and is written as:

$$G = \frac{[IC_{50}]^n}{[IC_{50}]^n + [C]^n} G^0 \quad (5)$$

where G and G^0 are the electrical conductance of a given ion channel with and without compound, respectively, $[IC_{50}]$ is the half-maximal inhibitory concentration of the cardiac sensitive compound that was determined from the results of IonWorks Quattro screening performed at AstraZeneca (Elkins et al., 2013; Mirams et al., 2014), $[C]$ is the compound concentration, and n is the Hill coefficient which was set to 1 (Mirams et al., 2014). The inhibition of five membrane ion channel currents were modeled in this study: rapid delayed inward rectifying K^+ current (I_{Kr}), slow delayed inward rectifying K^+ current (I_{Ks}), fast Na^+ current (I_{Na}), long lasting- or L-type Ca^{2+} current (I_{CaL}), and transient outward K^+ current (I_{to}). The effects of dofetilide and vardenafil concentration on the conductance of each channel are shown in Fig. 2. Dofetilide and vardenafil were used to illustrate the results of the application of a cardiac sensitive drug to the model. Nebivolol was also tested (see supplement) however, the effects of fibroblasts on the drug-induced proarrhythmia potential of any compound can be analyzed using $[IC_{50}]$s on each of the five defined ion channel current as described in Mirams et al. study (Mirams et al., 2014).

Since cardiomyocytes are outnumbered by fibroblasts in normal cardiac tissue by a ratio of 2 to 3 (Rohr, 2012), we simulated the cardiomyocyte action potential by coupling a single cardiomyocyte