Journal of Pharmacological and Toxicological Methods 68 (2013) 88–96

Contents lists available at SciVerse ScienceDirect

Journal of Pharmacological and Toxicological Methods

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

Original article

Evaluation of an in silico cardiac safety assay: Using ion channel screening data to predict QT interval changes in the rabbit ventricular wedge ☆

Kylie A. Beattie [a,*], Chris Luscombe [b], Geoff Williams [a], Jordi Munoz-Muriedas [b], David J. Gavaghan [a], Yi Cui [c], Gary R. Mirams [a]

[a] Computational Biology, Department of Computer Science, University of Oxford, Oxford, OX1 3QD, UK
[b] Computational Chemistry, GlaxoSmithKline, Stevenage, SG1 2NY, UK
[c] Safety Pharmacology, Safety Assessment, GlaxoSmithKline, Ware, SG12 0DP, UK

ARTICLE INFO

Article history:
Received 8 February 2013
Accepted 17 April 2013

Keywords:
Action potential
Cardiac safety
Compound screening
hERG
Ion channels
Mathematical model
Methods
QT interval
Rabbit ventricular wedge

ABSTRACT

Introduction: Drugs that prolong the QT interval on the electrocardiogram present a major safety concern for pharmaceutical companies and regulatory agencies. Despite a range of assays performed to assess compound effects on the QT interval, QT prolongation remains a major cause of attrition during compound development. In silico assays could alleviate such problems. In this study we evaluated an in silico method of predicting the results of a rabbit left-ventricular wedge assay. **Methods:** Concentration–effect data were acquired from either: the high-throughput IonWorks/FLIPR; the medium-throughput PatchXpress ion channel assays; or QSAR, a statistical IC_{50} value prediction model, for hERG, fast sodium, L-type calcium and KCNQ1/minK channels. Drug block of channels was incorporated into a mathematical differential equation model of rabbit ventricular myocyte electrophysiology through modification of the maximal conductance of each channel by a factor dependent on the IC_{50} value, Hill coefficient and concentration of each compound tested. Simulations were performed and agreement with experimental results, based upon input data from the different assays, was evaluated. **Results:** The assay was found to be 78% accurate, 72% sensitive and 81% specific when predicting QT prolongation (>10%) using PatchXpress assay data (77 compounds). Similar levels of predictivity were demonstrated using IonWorks/FLIPR data (121 compounds) with 78% accuracy, 73% sensitivity and 80% specificity. QT shortening (<−10%) was predicted with 77% accuracy, 33% sensitivity and 90% specificity using PatchXpress data and 71% accuracy, 42% sensitivity and 81% specificity using IonWorks/FLIPR data. Strong quantitative agreement between simulation and experimental results was also evident. **Discussion:** The in silico action potential assay demonstrates good predictive ability, and is suitable for very high-throughput use in early drug development. Adoption of such an assay into cardiovascular safety assessment, integrating ion channel data from routine screens to infer results of animal-based tests, could provide a cost- and time-effective cardiac safety screen.

1. Introduction

Drug interactions with cardiac ion channels can lead to lengthening of the QT interval on the electrocardiogram (ECG) (Grant, 2009; Yap & Camm, 2003). This phenomenon has been linked with the potentially lethal ventricular arrhythmia Torsades de Pointes (TdP) (Dessertenne et al., 1966; Pollard et al., 2010). Cardiovascular toxicity remains the main safety reason for the discontinuation of development and market withdrawal of compounds (Valentin, 2010). 21.4% of the withdrawn compounds from major international markets between 1990 and early 2012 were as a consequence of QT prolongation liability and/or association with TdP (Shah, 2012). Compound withdrawal not only implies that a risk to patient safety has been posed, but also results in a substantial loss of invested time, money and resources. It is thus important for pharmaceutical companies to identify compounds that prolong QT as early as possible during drug development (Laverty et al., 2011).

Often, compounds which prolong the QT interval inhibit the rapid component of the delayed rectifier potassium current I_{Kr} (encoded by human Ether-a-go-go Related Gene (hERG), whose protein forms the pore alpha-subunit of the I_{Kr} channel) (Sanguinetti, Jiang, Curran, & Keating, 1995). Due to its fundamental role in controlling repolarisation of the ventricular action potential, hERG channel block leads to a lengthening of the Action Potential Duration (APD) of a single cell, manifested as

Abbreviations: AP (D), Action Potential (Duration); ECG, electrocardiogram; ECVAM, European Centre for the Validation of Alternative Methods; FLIPR, FLuorescence Imaging Plate Reader; GSK, GlaxoSmithKline; hERG, human-Ether-a-go-go Related Gene; IC_{50}, Concentration for 50% Inhibition; ICH, International Conference for Harmonization; I_{Kr}, rapid delayed rectifier potassium current; I_{Ks}, slow delayed rectifier potassium current; QSAR, Quantitative Structure Activity Relationship; pIC_{50}, minus $\log_{10}$ of IC_{50}; TdP, Torsades de Pointes.

* Corresponding author. Tel.: +44 1865210588.
E-mail address: kylie.beattie@cs.ox.ac.uk (K.A. Beattie).

1056-8719/$ – see front matter © 2013 The Authors. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.vascn.2013.04.004

QT prolongation on the ECG. Thus, hERG channel block and QT prolongation are considered important factors in the detection of pro-arrhythmic liability (Pollard, Valentin, & Hammond, 2009). However, consideration of drug interactions with multiple ion channels is important for improved pro-arrhythmic risk prediction (Davies et al., 2012; Mirams et al., 2011; Obiol-Pardo, Gomis-Tena, Sanz, Saiz, & Pastor, 2011). Some pharmaceutical companies now routinely screen a selection of ion channels in addition to hERG, including sodium and L-type calcium channels to facilitate such investigations.

The International Conference for Harmonization (ICH) proposed the ICH S7B (Anon, 2005b) and E14 (Anon, 2005a) guidelines, which provide advice for preclinical and clinical assessment of drug-induced QT prolongation liability (Darpo, Nebout, & Sager, 2006). ICH S7B suggests that in vitro I_{Kr} and in vivo QT measurements are performed as standard during drug development. These tests typically comprise patch clamp experiments using a hERG expression system and a conscious animal QT study (typically in dog), but can non-exclusively include: rabbit and dog ventricular wedge studies, Langendorff heart preparations and in vivo studies in other animals (Pugsley, Authier, & Curtis, 2009). The ICH E14 suggests that a human thorough-QT trial is conducted during clinical development (Pollard et al., 2010; Recanatini, Poluzzi, Masetti, Cavalli, & De Ponti, 2004).

The ICH guidelines were intentionally written in an unrestrictive manner, enabling pharmaceutical companies to explore new in vivo, in vitro and in silico methodologies for acquiring QT measurements (Darpo, 2009; Picard et al., 2011). In silico assays have great potential, particularly in early drug development, to provide integrative, high-throughput, cost-effective and efficient solutions. Pharmaceutical companies are currently exploring such approaches (Mirams, Davies, Cui, Kohl, & Noble, 2012). Through integrated risk assessment, these approaches could complement existing safety tests and reduce the current use of animal-based experiments. An in silico approach, frequently enlisted within pharmaceutical companies, is Quantitative Structure Activity Relationship (QSAR) modelling which uses information regarding the chemical structure of compounds to infer properties of their biological activity (Inanobe et al., 2008).

Additional in silico approaches, developed in collaboration with, and having applicability to inform decisions within, pharmaceutical companies, include the work of Bottino et al. (2006), Mirams et al. (2011) and Davies et al. (2012). These approaches all use ion channel data acquired from routine high-throughput screens performed early in preclinical development, to infer results which would be of interest during later compound development. Davies et al. (2012) evaluate the ability of an in silico assay in predicting the results, and the associated inter-individual variability, of drug effects on canine APD in isolated myocytes. The assay demonstrates high levels of sensitivity and specificity.

The approach adopted in this study is similar. We evaluate the ability of an in silico action potential simulation assay, which uses concentration–effect data from high-throughput ion channel screens, to predict the results of the rabbit left-ventricular wedge assay. The rabbit wedge assay is performed during compound development at GlaxoSmithKline (GSK) and the preclinical data obtained has been found to correlate well with clinical outcomes (Joshi, Dimino, Vohra, Cui, & Yan, 2004; Liu et al., 2006). The number of compounds we include for evaluation in this study is much increased to that of Davies et al. (2012), strengthening the confidence in conclusions drawn. Compounds included belong to a wide variety of chemical and therapeutic categories. Additionally, we examine the use of ion channel data from three different stages of assay; (QSAR, IonWorks/FLIPR and PatchXpress). Simulated action potentials generated from single cell simulations, and pseudo-ECGs from one-dimensional tissue simulations, are compared to ECGs recorded in the rabbit ventricular wedge assay. The predictivity of the model is assessed when using the available ion channel data in a number of different ways. In addition to evaluating the assay's predictive capacity, the evaluation has allowed us to infer the most advantageous use of available ion channel data, and where in the drug development process such an in silico approach would be most beneficial.

2. Methods

2.1. Statement on use and care of animals

All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GlaxoSmithKline Policy on the Care, Welfare and Treatment of Animals.

2.2. Electrophysiology

2.2.1. Ion channel screens

Ion channel screens are performed on cell lines, based on Human Embryonic Kidney-293 (HEK-293) and Chinese Hamster Ovary (CHO) cells. The Molecular Devices PatchXpress 7000A assay was used to screen hERG, NaV1.5 and CaV1.2 channels. The Molecular Devices IonWorks Quattro assay was used to screen hERG, NaV1.5 and KCNQ1 channels. The Molecular Devices FLuorescence Imaging Plate Reader (FLIPR) assay was used to screen the CaV1.2 channel. CaV1.2 data from FLIPR was gathered alongside data from the IonWorks assay for the hERG, NaV1.5 and KCNQ1 channels.

Details of the cell culture and preparation process, the solutions prepared for ion channel screening and the controls used are described in Supplementary material S.3. Voltage protocols applied to obtain IC_{50} value estimates from the IonWorks and PatchXpress assays and the method of fluorescence measurement used in the FLIPR assay are also outlined. Details of the methodologies used for PatchXpress assay screening of the hERG and NaV1.5 channels can be found in Donovan et al. (2011). A curve of the form shown in Eq. (1) was fitted to the concentration-response data derived from the normalised data produced by each of the assays in order to determine IC_{50} values (and in some cases Hill coefficients).

2.2.2. QSAR models

QSAR models for hERG, NaV1.5 and CaV1.2 were created from available measured data from ion channel assays described in Section 2.2.1. The structures and measured responses were presented to an automated modelling software application (QSAR Workbench (Accelrys Inc.)). Models based upon Support Vector Machines (SVM) (Burbidge, Trotter, Buxton, Holden, et al., 2001) were developed for the hERG and NaV1.5 channels, whilst a random forest classifier (Svetnik et al., 2003) was selected for the CaV1.2 channel. The output from the SVM was a predicted pIC_{50} value for each compound. The output for the CaV1.2 channel was a predicted class which was then equated with a pIC_{50} value.

2.3. Rabbit ventricular wedge assay

Details of the preparation of rabbit left-ventricular wedge have been described previously in Liu et al. (2006). Briefly, female rabbits were sedated with 6 mg/kg xylazine (i.m.), anticoagulated with 800 U/kg heparin (i.v.) and anaesthetised with ketamine (30–35 mg/kg, i.v.), or with pentobarbital (50 mg/kg, i.v.). The left circumflex or anterior descending branch of the coronary artery of the excised rabbit heart was cannulated and perfused in cardioplegic solution. A transmural left-ventricular wedge was dissected and placed in a tissue bath and arterially perfused with Tyrode's solution. After approximately 1 h of equilibration in the bath at a stimulation frequency of 1 Hz, the stimulation frequency was reduced to 0.5 Hz for 5 min of stabilisation where the baseline ECG was measured. The preparations were then returned to a stimulation frequency of 1 Hz and perfused with Tyrode's solution containing a test compound. For each test compound concentration, the preparation was perfused for approximately 30 min at a frequency of 1 Hz followed by 5 min at a frequency of 0.5 Hz, where again the ECG was recorded.