Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmacological and Toxicological Methods 57 (2008) 9 – 22
Journal of Pharmacological and Toxicological Methods
www.elsevier.com/locate/jpharmtox

Original article

Impact of study design on proarrhythmia prediction in the SCREENIT rabbit isolated heart model

Jill V. Steidl-Nichols [a,*], Gilles Hanton [c], Joanne Leaney [b], Ruei-Che Liu [d], Derek Leishman [e], Aileen McHarg [a], Rob Wallis [b]

[a] Global Safety Pharmacology, Pfizer Global Research and Development, La Jolla, United States
[b] Global Safety Pharmacology, Pfizer Global Research and Development, Sandwich, England
[c] Drug Safety Research and Development, Pfizer Global Research and Development (Amboise), France
[d] Statistics, Pfizer Global Research and Development (La Jolla), United States
[e] Lilly Research Laboratories, Greenfield Laboratories, P.O. Box 708, Greenfield, IN 46140, United States

Received 25 September 2006; accepted 22 June 2007

Abstract

Introduction: Prediction of the propensity of a compound to induce Torsades de Pointes continues to be a formidable challenge to the pharmaceutical industry. Development of an *in vitro* model for assessment of proarrhythmic potential offers the advantage of higher throughput and reduced compound quantity requirements when compared to *in vivo* studies. A rabbit isolated heart model (SCREENIT) has been reported to identify compounds with proarrhythmic potential based on the observance of compound-induced triangulation and instability of the monophasic action potential (MAP), ectopic beats, and reverse-use dependence of prolongation of the MAP duration. Previous reports have indicated that this model qualitatively identifies proarrhythmic compounds and suggest the use of this model to assign safety margins for human clinical use. The intent of this series of studies was to evaluate the impact of study design on the proarrhythmic concentration predicted by this model. **Methods:** Nine compounds of varying proarrhythmic potential and a negative control were tested in a blinded fashion using a series of different experimental protocols: Compounds were tested at multiple concentration ranges and extended perfusion times were also evaluated. **Results:** In general when the dataset is viewed as a whole, the model did identify proarrhythmic compounds, however the concentration at which action potential prolongation, triangulation, instability, reverse-use dependence and ectopic beats occurred often varied based on the concentration range selected. Further analysis using extended compound perfusion times demonstrated that variability may be due in part to lack of adequate equilibration of compound with the cardiac tissue. **Discussion:** We report that the model correctly identified proarrhythmic agents in a qualitative manner, but that study design impacts the proarrhythmic concentration derived from the model.

Keywords: hERG; Methods; Proarrhythmia; QT prolongation; Safety margin; SCREENIT rabbit isolated heart model; Torsades de Pointes; TRIad

1. Introduction

Torsades de Pointes (TdP) is a life threatening ventricular arrhythmia associated with drug-induced inhibition of the hERG potassium channel and QT prolongation (Curran et al., 1995; Sanguinetti, Jiang, Curran & Keating, 1995; Haverkamp et al., 2000). However, not all compounds that inhibit the hERG current induce QT prolongation in humans (*e.g.* verapamil; Redfern et al., 2003). Moreover, it is recognized that while QT prolongation in humans is associated with TdP in a number of instances, not all patients who experience prolongation of the QT interval convert to TdP (Guanzon & Crouch, 2004). Most pharmaceutical companies now assess compound interactions and functional effects on the hERG current early in the drug discovery process in an attempt to predict the risk for arrhythmia and to avoid the liabilities associated with QT prolongation. Since there is a lack of complete understanding of the mechanism by which hERG current inhibition and

* Corresponding author. Global Safety Pharmacology, Pfizer Global Research and Development, La Jolla Laboratories, 10646 Science Center Drive, San Diego CA 92121, United States. Tel.: +1 858 526 4798; fax: +1 858 678 8290.
E-mail address: jill.steidl@pfizer.com (J.V. Steidl-Nichols).

1056-8719/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.vascn.2007.06.003

QT prolongation convert to TdP, companies are forced to rely on these experimental endpoints even though their capacity to predict TdP in human is thought to be suboptimal.

Numerous *in vitro* and *in vivo* proarrhythmia models (Akita et al., 2004; Antzelevitch, 2004; Aubert et al., 2006; Champeroux et al., 2005; Fossa et al., 2004; Milberg et al., 2004; Vos, Verduyn, Gorgels, Lipcsei & Wellens, 1995; Weissenburger et al., 1991) have been introduced in an attempt to address the gap between QT prolongation and induction of TdP. Development of a model to predict TdP in humans is a challenging task, as there are many different known and unknown factors that contribute to induction of the arrhythmia (*e.g.* bradycardia, electrolyte concentrations, genetic polymorphisms; Haverkamp et al., 2000; De Ponti, Poluzzi, Cavalli, Recanatini & Montanaro, 2002), and every model may not necessarily recapitulate all of these factors. Evaluation of the capacity of a model to predict TdP also poses a challenge since the actual incidence rate of clinical TdP is not well characterized due to the rarity of TdP, and the difficulty in attributing sudden death to the arrhythmia outside of a clinical setting.

One model that is currently under evaluation is the Hondeghem Pharmaceutical Consulting SCREENIT Langendorff-perfused rabbit isolated heart model (Hondeghem, Carlson & Duker, 2001). In this model, monophasic action potentials (MAPs) are recorded and in addition to MAP duration, compound induction of triangulation, reverse-use dependence and instability (abbreviated here as TRI) and ectopic beats are assessed. Numerous reports state that the model effectively differentiates proarrhythmic from safe compounds (Hondeghem, Lu, van Rossem, De Clerck, 2003; Hondeghem & Hoffman, 2003; Valentin, Hoffman, De Clerck, Hammond & Hondeghem, 2004), and suggest that concentrations tested in the model can be compared to free therapeutic concentrations in human for prediction of the potential to induce TdP as part of an integrated risk assessment (Valentin et al., 2004; Lawrence, Bridgland-Taylor, Pollard, Hammond & Valentin, 2006).

A model must be robust if it is to be used for determination of safety margins for human use. In order to further investigate the reliability of the model and the impact of study design, we submitted two blinded sets of standard compounds to Hondeghem Pharmaceutical Consulting for testing in the SCREENIT model. In the first set of experiments (round 1), by testing compounds using two overlapping concentration ranges we found that proarrhythmic concentration varied between the two concentration ranges. To clarify these results, the second round of experiments evaluated the effect of further increasing the number of concentration ranges tested, extension of perfusion time, and increasing the number of hearts tested. We report that the model correctly identified proarrhythmic agents in a qualitative manner, but that study design impacts the proarrhythmic concentration derived from the model.

2. Methods

Measurements were made by Hondeghem Pharmaceutical Consulting using the SCREENIT (version 7) system; an automated rabbit Langendorff set-up in which monophasic action potentials (MAPs) are measured (Hondeghem et al., 2001).

2.1. Cardiac tissue preparation

This investigation conforms with the *Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* (86/609/EEC). Following stunning, hearts were removed from female albino rabbits (~2.5 kg), washed free of blood and perfused at a constant pressure of 80 cm H_2O with solution (heated to 37 °C and gassed with 95% O_2/5% CO_2) of the following composition (in mM: NaCl 118, KCl 4, $NaHCO_3$ 22, $MgCl_2$ 1.1, NaH_2PO_4 0.4, $CaCl_2$ 1.8, dextrose 5, pyruvate 2, and creatine 0.038 mM, pH 7.35). The heart was opened at the level of the atria to expose the His bundle which was then sectioned. Two stimulating electrodes were positioned either side of the His bundle and recording electrodes were placed at the left ventricular epicardium and beneath the left ventricular septal endocardium. The reference electrode consisted of an isotonic KCl/1.8 mM $CaCl_2$-perfused 1 mm tube positioned at the left ventricular epicardium. Heart preparations were stimulated at 1.5 times the threshold stimulation current.

2.2. Electrophysiological measurements

The following parameters were measured as previously described (Hondeghem et al., 2001): action potential duration at 30%, 60% and 90% repolarization (APD_{30}, APD_{60}, APD_{90}), triangulation, reverse-use dependence, instability, and the number of ectopic beats as a measure of proarrhythmia. APD_{30}, APD_{60}, and APD_{90} were measured from the midpoint of the upstroke until 30%, 60% or 90% repolarization, respectively. APD was calculated in the presence of various concentrations of compound and compared to the APD during the baseline recording (prior to compound perfusion) in order to calculate percent change in APD. Triangulation was calculated as the difference between APD_{90} and APD_{30} and reported in milliseconds. Reverse-use dependence was quantified as the difference in APD_{60} between the first 10 and the last 20 MAPs in a train of 30 pulses at various cycle lengths (300, 400, 500 and 1000 ms). The difference was calculated in the presence of compound and compared to the difference in compound-free solution for each cycle length, and reported in milliseconds. Instability was characterized as the variation in APD_{60} between consecutive MAPs during the last 3 min of an experimental period. Upper and lower quartiles were calculated and the difference in milliseconds between these was reported as instability. EADs were defined as those which occurred 150 ms after the upstroke of the action potential with a minimal upstroke of 20 ms, a duration between 100 and 500 ms, and an amplitude <70% of that of the previous action potential.

Ectopic beats were defined as any MAPs in which the upstroke occurred >80 ms after the stimulus: Ectopics were classified as ventricular tachycardia (VT) if 6 or more MAPs of similar amplitude and upstroke velocity occurred in a time span of 3 s. Torsades de Pointes (TdP) was defined as at least 3 deflections of 3 oscillations per second with significant beat-to-beat variability in upstroke velocity, MAP amplitude and duration, and with fewer than 70% of the deflections reaching a diastolic potential close to that of the last preceding normal diastole. Random consecutive MAPs with no reproducible