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Original article

Use of arterially perfused rabbit ventricular wedge in predicting arrhythmogenic potentials of drugs

Xian Chen, Jason S. Cordes, Jenifer A. Bradley, Zhuoqian Sun, Jun Zhou *

Department of Safety Pharmacology, Worldwide Safety Sciences, Groton Laboratories, Pfizer Global Research and Development, Groton, CT 06340, USA

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Abstract

Introduction: A growing number of drugs have reportedly been associated with delayed ventricular repolarization and a potentially fatal but rare arrhythmia, torsade de pointes (TdP). There is obviously a call for a validated proarrhythmia model that distinguishes proarrhythmic drugs from nonarrhythmogenic drugs. **Methods:** In this article, we validated the arterially perfused rabbit left ventricular wedge preparation model and examined its use in predicting proarrhythmic potentials of drugs. A fairly detailed methodological description about this technically challenging model was given, aiming to help others establish the assay successfully. Parameters commonly used in the action potential studies were verified and critical experimental conditions (e.g. stability and reproducibility of recordings) were examined. Six commercially available compounds with various proarrhythmic potentials were administered in the model to evaluate their correlations with individual clinical outcomes. **Results:** Our study indicated that, in a successful experiment, the action potential duration (APD) can be stably maintained for several hours without intervention. Dofetilide, DL-sotalol, cisapride, risperidone and moxifloxacin increased endo- and epicardial APD_{90}, QT interval and T_{P-E} (peak-to-end time of the T wave) in a reverse use-dependent manner within clinically relevant concentration ranges. Phase 2 early afterdepolarizations (EADs) were observed at 1.6, 2.3, 16.7, 37.5 and 7.9 fold, respectively, their corresponding unbound therapeutic concentrations. In contrast, fluoxetine at up to 3 μM (~35 fold unbound therapeutic mean plasma concentration after 60 mg/day, p.o. for 5 weeks) had only a mild prolonging effect on APD_{90} and QT with essentially no effect on T_{P-E}. **Discussion:** Our results strongly support the usefulness of this model in predicting a compound's arrhythmogenic potential in humans within clinically relevant concentration ranges, and the experimental results with this model need to be interpreted in light of each drug's pharmacokinetic and pharmacodynamic behavior in clinic.

Keywords: Action potential (AP); Arrhythmia; Early afterdepolarization (EAD); Preclinical models; QT prolongation; Torsade de pointes (TdP)

1. Introduction

Drug-induced delayed ventricular repolarization (QT-interval prolongation) has reportedly been associated with the incidence of a rare but potentially lethal arrhythmia, torsade de pointes (TdP). Electrophysiological studies indicate that most QT-prolonging drugs exert their effects by inhibiting a delayed rectifier potassium channel, I_{Kr}, the major determinant of cardiac repolarization. However, I_{Kr} inhibition alone does not always translate into a proarrhythmic risk, as drugs like verapamil, amiodarone and fluoxetine, which possess I_{Kr}-blocking activities at clinically relevant concentrations, are not associated with TdP (Milberg et al., 2004; Redfern et al., 2003). Therefore, while it is still considered a "gold-standard" in vitro assay for the prediction of QT prolongation in man and mandated for every compound pre-IND by the ICH S7B guideline (http://www.ich.org), the I_{Kr} or hERG patch-clamp assay alone has limited value in predicting the arrhythmogenic potentials of drugs. Furthermore, there is plenty of evidence indicating that the QT interval prolongation (commonly expressed as QTc, after correction for heart rate changes) is also a poor predictor of TdP, although it has been considered as a surrogate marker (Antzelevitch & Shimizu, 2002; Belardinelli, Antzelevitch, & Vos, 2003; Thomsen et al., 2004). For instance, patients experiencing drug-induced TdP could even have the arrhythmia terminated by amiodarone while their QTc intervals were further prolonged (Antimisiaris et al., 1994; Mattioni et al., 1989; van de Loo, Klingenheben, & Hohnloser, 1994). Unfortunately, the current regulatory guidelines require both the I_{Kr} or hERG study and in vivo QT interval

* Corresponding author. Tel.: +1 860 715 2413; fax: +1 860 715 8093.
E-mail address: jun.zhou@pfizer.com (J. Zhou).

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measurements to be conducted prior to first-in-human (FIH) applications, and QT interval information (especially in clinical trials) has been heavily relied upon in determining labeling languages for a new drug. The lack of accurate prediction for arrhythmogenic potentials of drugs using current QT assessment strategies has significantly impacted the pharmaceutical industry. This is reflected in a drastically increased investment and discontinuation of programs or compounds because of a positive QT signal, which translates to reduced potential new therapies to the market (Fermini & Fossa, 2003). A commercial and therapeutic breakthrough in depression with an excellent cardiac safety profile, fluoxetine, for example, would unlikely be developed according to today's standards in preclinical QT assessments. Clearly, there is a need for arrhythmogenic models that are validated with both proarrhythmic and nonarrhythmic agents and, consequently, applied in the early developmental stages of drugs for better decision-making (Lawrence, Pollard, Hammond, & Valentin, 2005).

Although a number of preclinical models, both in vitro and in vivo, are reportedly available to specifically investigate the proarrhythmic potential of drugs in man, only a few are shown to hold a promising value in discriminating pro- from non-arrhythmic drugs (Lawrence et al., 2005). We chose to validate the arterially perfused rabbit left ventricular wedge preparation because of its ability to monitor the two major mechanisms that are believed to underlie TdP: early afterdepolarization (EAD)-mediated triggered activity and transmural dispersion of repolarization (TDR)-mediated reentry (Antzelevitch, 2004; Peters, Cabo, & Wit, 1999; Poelzing & Rosenbaum, 2005). Although this model has been reported for almost 10 years (Yan & Antzelevitch, 1996) and publications are available on its use in identifying the mechanism of arrhythmias for some QT-prolonging agents, technical difficulties in making a successful preparation are well recognized and have limited its use in the industry (Lawrence et al., 2005). Consequently, only a few academic laboratories have published their work with this preparation. Moreover, reports on the drug effect in this model are often limited to a single agent, and the experimental conditions and animal species are often inconsistent, making overall evaluation of the model somewhat difficult and, therefore, beckoning a "further independent validation" (Lawrence et al., 2005). In this study, we report a fairly detailed methodology on the rabbit wedge, aiming to help others overcome the difficulties of the technique based on our experience. We have also tested 6 commercially available compounds in the preparation and provided an evaluation on the value of the model in predicting proarrhythmic potentials of drugs.

2. Materials and methods

2.1. Surgical and cannulation procedures

The use of animals and the surgical procedures were approved by the Pfizer Institutional Animal Care and Use Committee. A New Zealand White rabbit (female, 2.5–5.5 kg) was restrained, sedated by intramuscular injection of xylazine at 5 mg/kg. Heparin was administered intravenously at 600–800 USP units/kg 10–15 min later. The animal was then anesthetized by i.v. injection of 30–35 mg/kg ketamine·HCl and placed on a surgical board. The chest was opened and the heart was removed promptly. The heart was then washed and placed in cold (~10 °C) 95% O_2–5% CO_2 saturated cardioplegic solution (in mM): 129 NaCl, 24 KCl, 0.9 NaH_2PO_4, 20 $NaHCO_3$, 1.8 $CaCl_2$, 0.5 $MgSO_4$, and 5.5 glucose.

The left circumflex branch of the coronary artery was then located, cut open with a sharp single-edged razor, and cannulated with a self-made PE cannulator of a proper size. Then the cardioplegic solution was allowed to perfuse the tissue and the cannulator was tied. After washing out the intravascular blood, a transmural left ventricular wedge from the anterior wall was dissected using a sharp single-edged razor blade. Unperfused areas, which may be easily identified from its reddish appearance because of the existence of unwashed erythrocytes, were carefully removed using the razor blade. The major leaking vessels were ligated, if necessary.

2.2. Cardiac action potential and transmural electrocardiogram (ECG) recording

The isolated ventricular wedge preparation was then placed in a tissue bath and arterially perfused with 36±0.5 °C Tyrode's solution (mmol/L): 129 NaCl, 4 KCl, 0.9 NaH_2PO_4, 20 $NaHCO_3$, 1.8 $CaCl_2$, 0.5 $MgSO_4$, and 5.5 glucose, pH 7.35 when buffered with 95% O_2 and 5% CO_2. The perfusion pressure was maintained at ~40 mm Hg and monitored through a pressure transducer connected with the PowerLab/8SP Data Acquisition System (ADInstruments Pty Ltd, Castle Hill, Australia). After being fixed with several stainless-steel needles on the silicon bottom, the tissue was paced with ~150% suprathreshold stimuli at 1 Hz through platinum bipolar electrodes that were in contact with the endocardial surface. Stimulation signals were generated by a DS8000 Digital Stimulator (World Precision Instruments, Inc., Sarasota, FL). Floating glass electrodes, prepared by using a P-97 Micropipette Puller (Sutter Instrument Company, CA), had a resistance of approximately 10–20 MΩ when filled with 2.7 M KCl. After connecting with the recording wire (Ag/AgCl), the end of the glass electrode was sealed with wax. Then the electrodes were manipulated using micromanipulators to penetrate the epicardial or endocardial myocardium, respectively. Action potentials from both sites were amplified through an IX2-700 Dual Intracellular Preamp (Dagan Corporation, Minneapolis, MN). The transmural electrocardiogram (ECG) was recorded by using two Ag/AgCl electrodes placed ~1 cm away from epicardial and endocardial surfaces and fed into an EX1 Differential Amplifier (Dagan Corporation, Minneapolis, MN). All the signals were monitored and recorded using the Chart 5 software (ADInstruments, Australia) through the PowerLab/8SP system (Fig. 1). An equilibrium period of at least 1 h was allowed in each experiment before any data collection. The preparations were maintained for up to 7 h post surgery. APD parameters were analyzed using the Peak Parameter extension within the Chart 5 program. From the transmural ECG recording, QT intervals were measured as the time between the beginning of the QRS complex and the end of the T wave. T_{P-E} was defined as the time difference