Journal of Pharmacological and Toxicological Methods 61 (2010) 238–250

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

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

Original article

The isolated rabbit heart and Purkinje fibers as models for identifying proarrhythmic liability

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ARTICLE INFO

Article history:
Received 27 July 2009
Accepted 20 January 2010

Keywords:
Action potential
Langendorff isolated heart
Preclinical models
Proarrhythmia
Purkinje fiber
QT prolongation
Rabbit

ABSTRACT

Introduction: Delayed ventricular repolarization is associated with rare, but often fatal, polymorphic tachyarrhythmias named Torsades de Pointes. ICH S7B guideline recommends an integrated approach for cardiovascular preclinical evaluation of new drug candidates, including action potential assays (as a Purkinje fiber test) but also proarrhythmia models. The aim of this preliminary study was to compare the respective value of two preclinical *in vitro* rabbit cardiac preparations—the Purkinje fiber and the isolated perfused heart (Langendorff method)—based on effects of dofetilide, a selective IKr inhibitor. **Methods:** Transmembrane action potentials from rabbit Purkinje fibers were recorded using a conventional intracellular glass microelectrode. Electrocardiograms from rabbit isolated hearts were evaluated for QRS, QT and T wave durations (Tpeak–Tend). The pacing protocol was the same for both preparations (basal rate of 80 bpm and pacing of 40, 60 and 140 bpm). Dofetilide was tested in both systems at concentrations of 1, 3 and 10 nmol/L. **Results:** In Purkinje fibers dofetilide induced a concentration- and reverse use-dependent increase in action potential durations measured at 50 and 90% of repolarization. At 10 nmol/L, only 3/10 fibers showed early after depolarizations. In the isolated heart model, dofetilide also induced a similar concentration- and reverse use-dependent increase in QT-interval. From 3 nmol/L, major changes in T wave morphology, R-on-T extrasystoles and TdP were observed, mainly at low rate. Prior to arrhythmias, T wave shape and duration were markedly altered suggesting an increase in the heterogeneity of cardiac ventricular repolarization. **Conclusions:** The effects of dofetilide were comparable in the two models for delayed repolarization but the isolated heart appears to be a better predictor for arrhythmias and a unique in vitro model to assess arrhythmogenic potential of QT prolonging compounds at least when associated with IKr/hERG inhibition.
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1. Introduction

Drug safety evaluation is critical in the development of new pharmaceutical entities to inform risk/benefit assessment (Pugsley et al., 2008). In a recent past, several drugs or drug candidates were either withdrawn from the market or suspended at a clinical stage because of their propensity to generate cardiac arrhythmias. One of the major drug-induced cardiac hazards is alteration of cell repolarization translating into QT interval lengthening. Indeed, prolongation of ventricular repolarization is associated with rare, but often fatal, polymorphic ventricular tachyarrhythmias, named Torsades de Pointes (TdP) which can degenerate into ventricular fibrillation. The increasing regulatory concern about drug-induced QT prolongation and TdP resulted in several recommendations and guidelines for preclinical and clinical risk assessment. After the original CPMP recommendations for adequate preclinical *in vitro* electrophysiological studies prior to first administration in humans, the ICH S7B guidelines proposed an integrated risk assessment paradigm based on both *in vitro* and *in vivo* testing and complemented on a need basis by follow-up mechanistic studies. In this integrated approach, action potential (AP) assays (as Purkinje fiber test) together with proarrhythmia models, are considered as critical for a comprehensive risk evaluation, besides compound tissue distribution and accumulation. These investigative studies are regarded as a key component of candidate development preclinical strategy because of the lack of univocal and quantitative link between QT prolongation and TdP occurrence. In other terms, QT interval prolongation is not believed to be a reliable indicator for proarrhythmic liability since full correlation between QT prolongation and occurrence of arrhythmias is not established.

Up to now, proarrhythmic risk was assessed during preclinical phase through an IKr assay (mainly a hERG assay) and a QT interval measurement, generally in a conscious non rodent species. Additional assays generally consisted in a Purkinje fiber test for evaluation of action potential duration. This *in vitro* model provides an overall picture of the effects of a drug candidate on all the ion channels contributing to AP genesis and therefore reflects part of the *in vivo*

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doi:10.1016/j.vascn.2010.01.011

conditions. Nevertheless, the Purkinje fiber model is a multicellular preparation constituted of a single cellular population expressing homogeneously the major ionic channels: in this respect this system does not reflect cardiac cell and tissue heterogeneity and does not allow therefore to fully judge for effects on the whole heart. Therefore, the Purkinje fiber test is only a surrogate model for the *in vivo* ECG and does not inform on spatial dispersion, heterogeneity and instability of repolarization. For this reason, there is a need for preclinical strategy refinement and enrichment through additional models aiming at predicting proarrhythmic risk beyond QT prolongation.

One of the models currently used for that purpose is the rabbit isolated heart, popularized through the SCREENIT system (Hondeghem et al., 2003). This system is a Langendorff-perfused female rabbit heart recording monophasic action potentials (MAP) from which several parameters are evaluated: action potential duration (APD), reverse use-dependence, instability and triangulation. One of the main benefits of this model is that data analysis is not restricted to AP prolongation but takes into account the overall shape of AP as well as stability of AP prolongation. MAPs provide a reasonable estimate for action potential duration at local transmural sites (El-Sherif et al., 1996; El-Sherif et al., 1997); however, despite MAPs are the only extracellular recording available in clinical practice, there is still a debate about the physical and biological significance of MAP versus transmembrane action potentials—particularly in terms of upstroke velocity, amplitude, movement artefacts, tissue depolarization by suction, pressure or KCl-tip electrode used for MAP recording. It was originally proposed that MAP originates from changes in membrane potential at the boundary of normal and depolarized cells around the depolarized electrode. More recently, Kondo and Kadish raised an alternative hypothesis that MAP actually originates from the non depolarized electrode (Kadish, 2004; Kondo et al., 2004).

The isolated arterially perfused rabbit left ventricular wedge preparation is another in vitro model currently available. This preparation is characterized by its long viability (as assessed through the stability of the electrophysiological parameters). Extracellular electrodes implanted in the endocardial and epicardial tissues generate a pseudo-ECG where QT interval changes, T wave morphology (as assessed through the duration between the peak and the end of the T wave) and arrhythmias can be monitored. The main value of this model is to provide electrophysiological basis to the ECG genesis through the contribution of different myocardial cells/tissues and to reflect for spatial dispersion of the electrical activity throughout the whole heart. This model generates high quality data, but the complexity of the technique prevents large scale routine use.

The Langendorff-perfused rabbit heart was implemented in order to circumvent possible misinterpretation of MAP data through an easily available *in vitro* preparation. In this model ventricular electrocardiograms are recorded directly through contact electrodes. In addition, the atrio-ventricular node is destroyed in order to allow heart rate pacing and optimization of QT data analysis by controlling heart rate throughout the experiment. This procedure does not therefore necessitate QT correction for heart rate variations, eliminates interferences with compound-induced changes in heart rate and allows exploring a wide range of heart rates (from 40 to 140 beats per min, bpm) thus modeling clinical situations including bradycardia.

The evaluation methodology for this model consisted in a head to head comparison of the effects of dofetilide in both the Langendorff-perfused rabbit heart and the rabbit Purkinje fiber.

Dofetilide was selected as a first step for this purpose because it selectively inhibits the rapidly-activating delayed rectifier potassium current IKr with a high affinity. A dissociation constant (Kd) of 3.9 nmol/L and an IC_{50} of 31.5 nmol/L were reported in isolated guinea-pig myocytes (Carmeliet, 1992; Jurkiewicz & Sanguinetti 1993). Dofetilide inhibits the human éther-à-go-go-related gene (hERG-channel) with an IC50 around 15 nmol/L (Rampe et al., 1997; Snyders & Chaudhary, 1996).

Furthermore, the effects of temperature on both preparations were also studied. The aim of these experiments was to compare non pharmacological repolarization lengthening in the two systems.

2. Methods

The animal care and experimental procedures for this study were compliant with the EEC directive 86/609/EEC (1986) and US Federal Guidelines (1985), Laboratory Animal Welfare Act. Furthermore, this study was performed in accordance to the standards of the ILAR Guide (1996) and was approved by an internal Ethics Committee.

2.1. Purkinje fibers

2.1.1. Preparation of the Purkinje fibers

Hearts from pre-pubertal New Zealand male rabbits (E.S.D.—Charles River Laboratories, 01400 Châtillon-sur-Chalaronne—France), weighing 1.3 to 1.8 kg and about 6–8 weeks old were quickly excised and placed into a cardioplegic solution with the following composition (in mmol/L): NaCl 120; KCl 27; $MgCl_2$ 1; NaH_2PO_4 1.8; $NaHCO_3$ 25; glucose 55; $CaCl_2$ 0.6; pH = 7.4. This solution was, maintained at room temperature and was continuously oxygenated with carbogen (95% O_2–5% CO_2). The heart was fixed in the organ bath for dissection. The left ventricle was opened and Purkinje fibers were carefully dissected out with a small piece of ventricular tissue to be pinned in the experimental chamber. There is a large variability in the distribution and morphology of the fibers within the left ventricle from one heart to another (for example, finest fibers were not used because of the instability of the impalement of the recording electrode). The Purkinje fibers, excised from the same left ventricular area were continuously superfused with the cardioplegic solution at a flow rate of 10 mL/min and a temperature of 37 ± 0.5 °C for about 20 min. The cardioplegic solution was then replaced by a normal physiological solution superfused at a flow rate of 10 mL/min and having the following composition (in mmol/L) : NaCl 120; KCl 4; $MgCl_2$ 1; NaH_2PO_4 1.8; $NaHCO_3$ 25; glucose 11; $CaCl_2$ 1.8; pH = 7.4 leading the fibers to repolarize under constant stimulation (about 120 bpm). The fibers were then left to stabilize for at least 1 to 2 h.

2.1.2. Electrophysiological measurements

Transmembrane action potentials were recorded using a conventional intracellular glass microelectrode, pulled with a micropipette puller (Narishige—type PE-2) and filled with KCl 3 mol/L. The microelectrode was connected to a microelectrode amplifier (Biologic—VF180). The resting membrane potential (RP), action potential amplitude (APA), maximal rate of action potential rise (Vmax) and action potential durations at 50 and 90% of repolarisation (APD50, APD90) were determined using a specific software (HEM-Notocord Systems, France). For each parameter, the mean was calculated during one minute at the end of each pacing period.

Exclusion criteria:

The preparation was discarded if one of the following parameters during the control period was out the following values: RP > −80 mV, Vmax < 500 V/s, APA < 120 mV, APD90 < 190 ms or > 350 ms. Over all the fibers used in this study and whatever the pacing rate, there were no spontaneous action potentials and the recording microelectrode did not dislodge along the control and the compound application periods.

2.1.3. Study design

The fibers (one/rabbit) were stimulated at a basal rate of 80 bpm. After a 45-min control period (each fiber served as its own control), dofetilide was tested at raising concentrations (1, 3 and 10 nmol/L), applied sequentially for 45 min each. Between the 30th and the 41st

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