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Original article

Comparison of guinea-pig ventricular myocytes and dog Purkinje fibres for in vitro assessment of drug-induced delayed repolarization

Derek A. Terrar [a,*], C.M. Wilson [b], S.G. Graham [b], S.M. Bryant [a], B.M. Heath [b]

[a] *University Department of Pharmacology, Mansfield Road, Oxford, OX1 3QT, United Kingdom*
[b] *Department of Safety Pharmacology, GlaxoSmithKline, The Frythe, Welwyn, Herts, AL6 9AR, United Kingdom*

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Abstract

Introduction: QT interval prolongation and Torsade de Pointes (TdP) arrhythmias are recognised as a potential risk with many drugs, most of which delay cardiac repolarization by inhibiting the rapidly activating K^+ current (I_{Kr}). The objective of this study was to compare the effects of compounds on cardiac action potentials recorded from guinea-pig ventricular myocytes and dog Purkinje fibres. **Methods and results:** Effects of dofetilide, sotalol, cisapride, terfenadine, haloperidol and sparfloxacin, compounds known to cause QT prolongation (positive controls), and nifedipine and verapamil, not associated with QT prolongation (negative controls) were studied on intracellular action potentials recorded from guinea-pig isolated ventricular myocytes (VM) and dog isolated Purkinje fibres (PF). Prolongation of action potential duration (APD) by sotalol, dofetilide and sparfloxacin was concentration-dependent and of greater magnitude in dog PF compared to guinea-pig VM. The maximum prolongation of APD in guinea-pig VM at 0.5 and 1 Hz was ~25% and this was associated with complete inhibition of I_{Kr} by dofetilide. Effects on APD of cisapride and haloperidol in both preparations, and terfenadine in guinea-pig VM, were biphasic, consistent with inhibition of multiple ion channels. There was no effect of terfenadine on APD in dog PF. Haloperidol increased APD by more than 25% in guinea-pig VM, consistent with effects on additional repolarizing currents. The negative controls shortened APD to a greater extent in guinea-pig VM compared to dog PF. In general, the positive control drugs increased action potential triangulation ($APD_{40–90}$) to a greater extent than APD_{90}. **Conclusion:** Guinea-pig isolated VM may be more sensitive for detecting APD prolongation with compounds inhibiting multiple ion channels and action potential triangulation ($APD_{40–90}$). Effects on repolarizing currents other than I_{Kr} were also distinguished in guinea-pig VM.

Keywords: Action potential; Arrhythmia; Cardiac; Dog; Guinea-pig; Purkinje fibre; QT interval; Repolarization; Torsade de Pointes; Ventricular myocytes

1. Introduction

Prolongation of the QT interval and Torsade de Pointes (TdP) arrhythmias are recognised as a potential risk with many drugs, most of which delay cardiac repolarization by inhibiting the rapidly activating potassium current (I_{Kr}) in the heart (De Ponti, Poluzzi, Cavalli, Recanatini, & Montanaro, 2002). The possibility of cardiac toxicity of new therapeutic agents has received particular attention since the publication of the effects of terfenadine on cardiac rhythm (Pinney, Koller, Franz, & Woosley, 1995; Salata et al., 1995) and the need for routine testing of new therapeutic agents on cardiac electrical activity has been extensively discussed in the ICH S7B guideline (Anon, 2005).

The cardiac Purkinje fibre is a well established preparation commonly used in the pharmaceutical industry to evaluate the cardiac electrophysiological effects of drugs known to prolong QT interval. Recent work has shown that the species plays an important role in the response of Purkinje fibres to drugs known to cause long QT, and whilst fibres from rabbit and dog have been shown to be suitable for this purpose, Purkinje fibres from other species, such as sheep and guinea-pig, were shown to be less sensitive to compounds known to prolong QT interval and failed to demonstrate reverse-use dependence in response to exposure to dofetilide and quinidine (Lu, Marien, Saels, & De Clerk, 2001). However, both ethical and financial consideration may limit the use of dogs for these studies.

* Corresponding author. Tel.: +44 1865 271613; fax: +44 1865 271853.
E-mail address: Derek.terrar@pharm.ox.ac.uk (D.A. Terrar).

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The sensitivity of in vitro preparations to compounds which affect cardiac repolarisation may not only depend on the species, but on the nature of the preparation. Multicellular in vitro preparations may present a significant barrier to the diffusion of compounds to the cardiac myocytes from which intracellular action potential are being recorded, an issue noted previously with multicellular preparations such as rabbit PF and guinea-pig papillary muscles (Cavero, Mestre, Guillon, Heuillet, & Roach, 1999; Hayashi et al., 2005). The use of cardiac isolated myocytes may be one way to overcome this problem.

Much less is known about the suitability of isolated ventricular myocytes for detection of drug-induced cardiotoxicity. Recent systematic studies, organised by ILSI-HESI and the JPMA, comparing the utility of nonclinical models to detect compounds which can delay repolarisation have not included cardiac isolated myocytes in their assessment, but have focussed on guinea-pig papillary muscles and dog PF as potential in vitro repolarisation models (Omata, Kasai, Hashimoto, Hombo, & Yamamoto, 2005; Hanson et al., 2006). Of the small mammals routinely used for cardiac muscle research, guinea-pig can be argued to most resemble man, certainly in preference to rat (which has a short action potential with a 'plateau' at an unusually negative potential of approximately −60 mV), and arguably in preference to rabbit (which shows a preponderance of the rapidly activating component of the delayed rectifier potassium current (I_{Kr}) with an unusually small contribution from the slowly activating delayed rectifier potassium current (I_{Ks}, Howarth, Levi, & Hancox, 1996). The electrical characteristics of myocytes from rat and guinea-pig are compared in Mitchell, Powell, Terrar and Twist (1987). Among the advantages of single myocytes over other isolated preparations are avoidance of drug effects on other cell types and rapid access of drugs to the cells of interest. On this basis, ventricular myocytes from the guinea-pig were chosen for comparison to the dog Purkinje fibre for this study.

Drug-induced prolongation of action potential duration (APD), particularly APD at 90% repolarisation (APD_{90}), has typically been used as a measure of the potential of compound to prolong QT interval, however recent evidence suggests that measurements such as triangulation (difference between APD_{40} and APD_{90}; $APD_{40–90}$) might also be useful (Hondeghem, 1994; Hanson et al., 2006; Kii et al., 2005). In this study, the sensitivity and specificity of these two parameters was studied by comparing the effects of the compounds on APD_{90} and on triangulation.

The objective of this study was to evaluate the effects of 8 compounds on intracellularly recorded cardiac action potentials in guinea-pig isolated ventricular myocytes (VM) and dog isolated Purkinje fibres (PF), electrically stimulated at 1 and 0.5 Hz under similar experimental conditions. The drugs selected for this study consist of positive and negative controls for TdP and QT prolongation and were selected to provide a range of effects. Dofetilide and sotalol are class III antiarrhythmic drugs frequently used as reference compounds and are expected to prolong action potential duration (Gwilt et al., 1991; Carmeliet, 1985); sparfloxacin, an antibiotic from the fluoroquinolone family, inhibits hERG and prolongs action potential duration, (Adamantidis, Dumotier, Caron, & Bordet, 1998; Bischoff et al., 2000; Kang et al., 2001; Patmore, Fraser, Maire, & Templeton, 2000); nifedipine and verapamil are known to inhibit calcium channels and are expected to shorten action potential duration (Rosen, Ilvento, Gelband, & Merker, 1974; Dangman & Hoffman, 1980); terfenadine, haloperidol and cisapride inhibit hERG, but also have actions on other cardiac ion channels and therefore may have mixed effects on action potential duration (Ming & Nordin, 1995; Hondeghem & Hoffmann, 2003; Hondeghem, Lu, van Rossem, & De Clerck, 2003).

2. Materials and methods

2.1. Isolation of myocytes and Purkinje fibres

All animals were treated in accordance to UK Home Office regulations (Animals (Scientific Procedures) Act 1986: London: Her Majesty's Stationery Office 1986) and the work was approved by internal ethical review. Myocytes were isolated enzymatically from guinea-pig ventricle as previously described (Powell, Terrar, & Twist, 1980). Briefly, male guinea-pigs were killed by cervical dislocation following stunning. Myocytes were isolated after perfusion of the heart with a physiological salt solution containing reduced calcium and 0.8 mg/mL of collagenase Type 1 (Worthington Biochemicals). Cells were stored at room temperature in Dulbecco's MEM (Life Technologies, Scotland) and used for electrophysiological investigation on the day of preparation.

Beagle dogs of either sex were killed on each experimental day by an intravenous overdose of sodium pentobarbitone (200 mg/kg). Free running ventricular PF were isolated from the heart and stored in gassed physiological salt solution (PSS) on ice prior to use.

2.2. Solutions

Both VM and PF were superfused with PSS containing (mM): NaCl 125, $NaHCO_3$ 25, KCl 5.4, NaH_2PO_4 1.2, $MgCl_2$ 1.0, $CaCl_2$ 1.8, glucose 5.5 (pH 7.4, oxygenated with 95% O_2/5% CO_2). Experiments in both preparations were carried out at approximately 36 °C.

Action potentials were recorded from dog isolated PF using conventional glass microelectrodes filled with 3 M KCl (resistance 10–60 MΩ). This, however, is not a suitable filling solution for making recordings from single ventricular myocytes, since chloride loading will take place resulting in 'blebbing' of the cell membrane as a result of activation of volume-sensitive ion channels. Therefore, action potentials were recorded from guinea-pig isolated myocytes with conventional glass microelectrodes filled with 1 M $KMeSO_4$ and 10 mM KCl using an Axoclamp 2A (Axon Instruments Inc) amplifier. Electrode resistance was in the range 40 to 60 MΩ.

2.3. Compounds and concentrations studied

The compounds studied consisted of the following six compounds associated with QT prolongation and/or TdP (positive controls; Redfern et al., 2003): dofetilide (supplied by GSK), dl-sotalol (Sigma), sparfloxacin (supplied by GSK), terfenadine (Sigma), cisapride (RDI) and haloperidol (Sigma), and two