



Assessment of drug-induced proarrhythmia: The importance of study design in the rabbit left ventricular wedge model



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ABSTRACT

In the present study, we investigated an impact of the stimulation rate on the detection of the proarrhythmic potential of 10 reference compounds with effects on different cardiac ion channels in the isolated arterially-perfused rabbit left ventricular wedge preparation. The compounds were tested in the wedge model using two distinct protocols; including baseline stimulation at 1-Hz followed by a brief period at 0.5-Hz, either without an additional brief period of 2-Hz stimulation (i.e. Protocol 1) or with 2-Hz stimulation (i.e. Protocol 2). As expected, QT-prolonging drugs (ibutilide and quinidine) prolonged the QT interval, similarly increased the Torsades de Pointes (TdP) score, and elicited early afterdepolarizations (EADs) in both protocols. HMR1556 and JNJ-303 (I_{Ks} blockers) also prolonged the QT interval up to 1 μ M similarly in both protocols. Nifedipine (Ca^{2+} antagonist) shortened the QT interval, and reduced force of contraction similarly in both protocols. However, Na^+ channel blockers (Ia, Ib, Ic) widened the QRS duration more in Protocol 2 than in Protocol 1. Furthermore, it was only possible to detect non-TdP-like ventricular tachycardia/fibrillation (VT/VF) induced by Na^+ blockers and by QT-shortening drugs (levcromakalim and mallotoxin) using the 2-Hz stimulation (Protocol 2).

Our data suggest that the inclusion of a brief period of fast stimulation at 2 Hz is critical for detecting drug-induced slowing of conduction (QRS widening), QT shortening and associated (non-TdP-like) VT/VF, which are distinct from the QT prolongation/TdP proarrhythmia in isolated, arterially-perfused rabbit left ventricular wedges.

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1. Introduction

The isolated arterially perfused rabbit left ventricular wedge model is widely appreciated as a sensitive *in vitro* model for evaluation of drug-induced cardiac electrophysiological changes, and prediction of arrhythmogenic potential (Gupta et al., 2008; Liu et al., 2006; Lu et al., 2010; Yan et al., 2001). Many factors may influence drug-induced electrophysiological changes in *in vitro* models. These factors include gender (Lu, Remeysen, Somers, Saels, & De Clerck, 2001b), temperature (Kiyosue, Arita, Muramatsu, Spindler, & Noble, 1993), species (Lu, Mariën, Saels, & De Clerck, 2001a), choice of the cardiac tissue (Lu, Vlamincx, & Gallacher, 2008a) and stimulation protocols/pacing rate of cardiac tissues (Lu, Vlamincx, Van Ammel, & De Clerck, 2002; Viswanathan, Shaw, & Rudy, 1999). To our knowledge, comparative studies of drug-induced responses to different classes of ionic current blockers or activators using different stimulation protocols are sparse in this model.

Over the last 20 years there has been a great regulatory focus on the identification of hERG (I_{Kr}) channel blockers and screening to remove

these properties from new molecular entities (NME's) heading towards clinical development. This industry de-risking screening paradigm was a reaction to the positive association of non-cardiovascular therapies with hERG-QT prolonging properties causing TdP's in man, resulting in removal from the market or restrictive labeling of a large number of marketed drugs (Kannankeril, Roden, & Darbar, 2010). However, the focus caused by these catastrophic TdP events meant that other off-target cardiovascular drug properties were ignored or forgotten. In the present study, we used a variety of drugs with differing electrophysiological mechanisms of action including Na^+ channel blockers (Vaughan-Williams Classes Ia, Ib, Ic); Ca^{2+} channel blockers; blockers of the rapidly (I_{Kr}) and slowly (I_{Ks}) activating delayed rectifier potassium channel; an ATP-sensitive K^+ channel (I_{KATP}) opener and an I_{Kr} activator, to determine cardiac electrophysiological changes and the proarrhythmic potential. This was done by using two different stimulation protocols in the isolated arterially perfused rabbit left ventricular wedge model. The difference between the protocols was the addition of a brief period of stimulation at 2 Hz (i.e. Protocol 2). Our results indicate that the stimulation protocol using different pacing frequencies indeed has a critical role in detecting drug-induced effects on cardiac electrophysiology, and the identification of risks for cardiac arrhythmias (VT/VF) that are distinct from drug-induced long QT and its risk for Torsades de Pointes (TdP).

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2. Materials and methods

2.1. Preparations

The methods used for isolation, perfusion, and recording of transmembrane activity from the arterially perfused ventricular wedge preparations, as well as for evaluating the viability and electrical stability of the preparation, have been described previously (Liu et al., 2006, 2012; Lu et al., 2010; Yan et al., 2001). Briefly, male and female rabbits weighing 2.5–3 kg were anticoagulated with heparin and anesthetized with ketamine (40–50 mg per kg IV). The chest was opened via a left thoracotomy, and the heart was excised and placed in a cardioplegic solution consisting of cold (4 °C) normal Tyrode's solution. Transmural wedges with dimensions of approximately 1.5 cm wide and 2–3 cm long were dissected from the left ventricle. The wedge tissue was cannulated via the left anterior descending artery or the circumflex artery and perfused with the cardioplegic solution. The preparation was then placed in a small tissue bath and arterially perfused with warm Tyrode's solution containing 4 mM K⁺ buffered with 95% O₂ and 5% CO₂ (T: 35.7 ± 0.1 °C, perfusion pressure: 40–50 mm Hg). The ventricular wedge was allowed to equilibrate in the tissue bath until electrically stable (usually one hour). The preparation was paced at 1 Hz throughout the study except as noted.

Transmembrane action potentials in wedge preparations were recorded simultaneously from epicardial and endocardial sites using 2 separate intracellular floating microelectrodes. A transmural ECG signal was recorded using extracellular silver/silver chloride electrodes, which were placed in the Tyrode's solution bathing the preparation near the epicardial and endocardial surfaces, along the same vector as the transmembrane recordings.

On the ECG, transmural dispersion of repolarization (TDR) is approximately equal to the interval from the peak to the end of the T wave (T_p–T_e), also expressed as rTp–Te (Tp–Te / QT * 100) (Gupta et al., 2008; Yan, Shimizu, & Antzelevitch, 1998). The QT interval was defined as the time from the onset of the QRS to the point at which the final downslope of the T wave crosses the isoelectric line. The TdP risk score system (from –2 to +15) was used to estimate the relative TdP risk of a test compound by taking into account the effects on QT interval, rTp–Te (Tp–Te/QT ratio which reflects the potential for phase 2 early afterdepolarization [EAD] development in the endocardium), and the incidence of Phase 2 EAD or TdP (Liu et al., 2006) (see Table 1). The incidence of EADs (Lu et al., 2002), Torsades de Pointes (TdP) (Yan et al., 2001), ventricular tachycardia (VT; defined as a run of four or more consecutive ventricular extra beats) and ventricular fibrillation (VF) according to Lambeth Convention (Curtis et al., 2013), and inexcitability (preparation not following stimulation) was recorded if observed during the experimental period.

In some experiments, isometric contractile force (FC) was measured by fixing one end of the preparation to the tissue chamber via a silk wire and the other end connected via a silk wire to a force transducer (Kent Scientific Corporation, Torrington, USA). Signals were digitized with an A-D converter (CED, England). All ECG parameters and FC were measured at a stimulation rate of 0.5 Hz. QRS rate dependence was calculated as the difference of the QRS duration at two stimulation rates: in

Protocol 1: QRS rate dependence = QRS duration at 1 Hz minus QRS duration at 0.5 Hz, and in Protocol 2: QRS rate dependence = QRS duration at 2 Hz minus QRS duration at 0.5 Hz.

2.2. Experimental protocols (Fig. 1)

Preparations were stimulated with 1–2 ms pulses at twice diastolic threshold applied to the endocardial surface via a bipolar silver electrode insulated except at the tips. In the first stimulation protocol (Fig. 1: upper part of the figure; Protocol 1 or standard protocol) (Liu et al., 2006), the preparation was paced at 1 Hz for 20 min then 0.5 Hz for 10 min before each measurement. In the second protocol (Fig. 1: lower part of the figure; Protocol 2), a brief 60-second period of faster pacing at 2 Hz (red bar) was introduced at the end of the 0.5-Hz period. The purpose of faster pacing was to examine use-dependent conduction delay and the resultant incidence of VT/VF. During experiments in which QRS duration increased more than 10 ms at 0.5 Hz, acceleration of the pacing rate was performed until marked conduction delay and VT/VF were observed. However, the faster pacing period was limited to 60 s at 2 Hz to 2.5 Hz in order to avoid myocardial ischemia in Protocol 2.

The following 10 compounds were tested using both stimulation protocols (mechanism; concentration range): ibutilide (Class III antiarrhythmic; 0.1, 1, 10 and 100 nM); quinidine (mixed Na⁺ and I_{Kr} blocker; 0.01, 0.1, 1 and 10 μM); ajmaline (Class Ia; 0.01, 0.1, 1 and 10 μM); lidocaine (Class Ib; 0.1, 1, 10 and 100 μM); flecainide (Class Ic; 0.3, 1, 3 and 10 μM); levcromakalim (K_{ATP} opener; 0.01, 0.1, 1 and 10 μM); mallotoxin (also called rottlerin; I_{Kr} activator; 0.01, 0.1, 1 and 10 μM); nifedipine (Ca²⁺ antagonist; 0.01, 0.1, 0.3 and 1 μM); and HMR1556 and JNJ-303 (I_{Ks} blockers; 0.01, 0.1, 1 and 10 μM). Each compound and stimulation protocol was tested in 6 to 7 preparations. Solvent (dimethyl sulfoxide; DMSO at 0.1%) was also tested in separate control groups for each drug using each stimulation protocol.

2.3. Compounds

JNJ-303, ibutilide and ajmaline were synthesized by Janssen Pharmaceutica NV (Beerse, Belgium), levcromakalim was from Tocris Cookson and other compounds were all purchased from Sigma. All compounds were ordered via Janssen's logistics department as dry neat, shipped to the test site, where they were manually dissolved (Heart Rhythm Solution–HRS, USA). The compounds were not blinded to the test site until the end of the study, but each test compound was coded with its respective JNJ-number and molecular weight. All compounds were then dissolved in dimethyl sulfoxide (DMSO, final maximal bath concentration up to 0.1%) at HRS. Compound was ordered based on the most recent batch in the ordering computer system and its availability with or without salt formulation, with molecular weight based on the batch weight of the ordered compound. The concentrations of test compounds were selected based on their IC₅₀ or EC₅₀ value for its respective ionic current blocking or activating activities reported in the literature (Table 2).

Table 1

A scoring system to estimate the relative TdP risk of a test compound using the isolated rabbit left ventricular wedge preparation (TdP score).

ΔQT interval %	–1	0	1	2	3
	<0%	0% to ≤5%	> 5% to ≤20%	>20% to ≤50%	>50%
ΔTp–Te/QT ratio (%)	–1	1	2	4	6
	≤5%	>5% to ≤10%	> 10% to ≤30%	>30% to ≤50%	>50%
Phase 2 EAD		1	2	4	6
		± EAD	EAD without “R-on-T”	EAD with “R-on-T”	TdP

Point scores for degree of change in QT interval and Tp–Te/QT ratio, and incidence of Phase 2 EAD or TdP are added for a maximum score of 15. Basic cycle length (BCL) = 2000 ms.

EADs: early afterdepolarizations; Tp–Te: Tpeak to Tend interval; TdP: Torsades de Pointes.

Adapted from publication by Liu et al., 2006.

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