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Research article

# New in vitro model for proarrhythmia safety screening: $I_{Ks}$ inhibition potentiates the QTc prolonging effect of $I_{Kr}$ inhibitors in isolated guinea pig hearts

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ABSTRACT

*Introduction:* Preclinical in vivo QT measurement as a proarrhythmia essay is expensive and not reliable enough. The aim of the present study was to develop a sensitive, cost-effective, Langendorff perfused guinea pig heart model for proarrhythmia safety screening.
*Methods:* Low concentrations of dofetilide and cisapride (inhibitors of the rapid delayed rectifier potassium current, $I_{Kr}$) were tested alone and co-perfused with HMR-1556 (inhibitor of the slow delayed rectifier potassium current, $I_{Ks}$) in Langendorff perfused guinea pig hearts. The electrocardiographic rate corrected QT (QTc) interval, the $T_{peak}$–$T_{end}$ interval and the beat-to-beat variability and instability (BVI) of the QT interval were determined in sinus rhythm.
*Results:* Dofetilide and HMR-1556 alone or co-perfused, prolonged the QTc interval by 20 ± 2%, 10 ± 1% and 55 ± 10%, respectively. Similarly, cisapride and HMR-1556 alone or co-perfused, prolonged the QTc interval by 11 ± 3%, 11 ± 4% and 38 ± 6%, respectively. Catecholamine-induced fast heart rate abolished the QTc prolonging effects of the $I_{Kr}$ inhibitors, but augmented the QTc prolongation during $I_{Ks}$ inhibition. None of the drug perfusions increased significantly the $T_{peak}$–$T_{end}$ interval and the sinus BVI of the QT interval.
*Discussion:* $I_{Ks}$ inhibition increased the QTc prolonging effect of $I_{Kr}$ inhibitors in a super-additive (synergistic) manner, and the QTc interval was superior to other proarrhythmia biomarkers measured in sinus rhythm in isolated guinea pig hearts. The effect of catecholamines on the QTc facilitated differentiation between $I_{Kr}$ and $I_{Ks}$ inhibitors. Thus, QTc measurement in Langendorff perfused guinea pig hearts with pharmacologically attenuated repolarization reserve and periodic catecholamine perfusion seems to be suitable for preclinical proarrhythmia screening.



## 1. Introduction

Torsades de pointes (TdP) is a rare, but life-threatening ventricular tachyarrhythmia, which is most often caused by the proarrhythmic activity of drugs. Several cardiac and non-cardiac drugs can induce TdP by blocking the rapid component of the delayed rectifier potassium current ($I_{Kr}$), leading to the prolongation of the action potential duration (APD) (Farkas & Nattel, 2010).

Newly developed drugs have to undergo cardiac safety screening in order to identify agents that have proarrhythmic activity. For this reason, pharmaceutical industry use preclinical models e.g. the hERG channel assays and in vivo QT measurements according to ICH S7B guideline (ICH, 2005). Unfortunately, in vivo QT measurement is expensive and not sensitive and specific enough (Guth, 2007). Therefore, less expensive, medium throughput models (e.g. isolated, Langendorff perfused heart models) with high sensitivity and specificity would be useful for proarrhythmia safety screening.

The activity of multiple potassium channels maintains normal repolarization of cardiac muscles under different circumstances, i.e.

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the block of one single type of potassium channel does not necessarily lead to proarrhythmia, due to the compensational activity of other potassium currents ("repolarization reserve") (Roden, 1998). However, multiple potassium channel inhibition makes the myocardium more sensitive to arrhythmias due to the impaired repolarization reserve (Lengyel, Varró, Tábori, Papp, & Baczkó, 2007). Recently, we have set up an isolated, Langendorff perfused rabbit heart model with pharmacologically attenuated repolarization reserve for proarrhythmia screening (Orosz et al., 2014). In the newly developed model, repolarization reserve is attenuated by pharmacological inhibition of the slow component of the delayed rectifier $K^+$ current ($I_{Ks}$), and the proarrhythmic liability of the test drug is assessed by determining the sensitive TdP biomarkers, the 'absolute' beat-to-beat variability and instability (BVI) parameters of the QT interval, i.e., determining the BVI parameters of the QT interval measured irrespective of the rhythm, even during arrhythmias (Orosz et al., 2014).

Since guinea pig myocardium has abundant $I_{Ks}$, remarkable decrease in repolarization reserve can be achieved with $I_{Ks}$ inhibition (Jost, Papp, & Varró, 2007). Also, the guinea pig heart is smaller than the rabbit heart, thus it needs less perfusion solution with less amount of the test drug, which may make the isolated, Langendorff perfused guinea pig heart more practical and less expensive than the isolated rabbit heart in drug-safety investigations. Therefore, the aim of the present study was to set up an isolated, Langendorff perfused guinea pig heart model for preclinical proarrhythmia safety screening. Thus, the effects of $I_{Kr}$ inhibitors on TdP biomarkers derived from ECG intervals were tested in isolated guinea pig hearts with attenuated repolarization reserve achieved by pharmacological inhibition of $I_{Ks}$. As occurrence of arrhythmias complicates ECG interval measurements, low concentrations of the $I_{Kr}$ inhibitor test drugs were applied in order to avoid frequent occurrence of arrhythmias and to allow ECG interval measurement in sinus rhythm.

## 2. Methods

### 2.1. Animals

Female guinea pigs (approximately 4-week-old, weighing 270–410 g) were utilized for the experiments as female gender may increase the susceptibility to drug-induced TdP (Liu et al., 1999; Lu, Remeysen, Somers, Saels, & De Clerck, 2001). Animals were acclimatized at the site for at least 3 to 4 days before any experiments started. Animals were kept under standard conditions (temperature 21 °C; relative humidity 55–65%; 12:12 h dark/light cycle) on commercial laboratory chow and tap water ad libitum. Animal maintenance and research were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All procedures using animals were approved by the local ethics committee (including the Ethical Committee for the Protection of Animals in Research at the University of Szeged, Hungary) and conformed to the rules and principles of the 86/609/EEC Directive.

### 2.2. General methods

The method of Langendorff perfusion we used was described in detail (Farkas & Coker, 2002; Farkas et al., 2006; Takács et al., 2015). Briefly, the animals were anticoagulated with sodium heparin (1000 international units) injected intraperitoneally and over-anaesthetized with thiopenthal (~3.2 ml/animal, ~470 mg/kg intraperitoneally). The heart was rapidly removed via thoracotomy and rinsed in ice-cold modified Krebs–Henseleit buffer solution containing (in mM): NaCl 118.5, glucose 11.1, $MgSO_4$ 0.5, $NaH_2PO_4$ 1.2, KCl 3.0, $NaHCO_3$ 25.0, $CaCl_2$ 1.8. The aorta was cannulated and hung on a Langendorff apparatus. The hearts were retrogradely perfused at a constant temperature of 37 °C with the modified Krebs–Henseleit buffer solution described above. A mixture of 95% $O_2$ and 5% $CO_2$ was bubbled through the buffer, which was equilibrated to pH 7.4. The perfusion pressure was maintained constant at 65 mm Hg. Volume-conducted electrocardiogram (ECG) was recorded by using National Instruments data acquisition hardware (PC card, National Instruments, Austin, TX., U.S.A.) and SPEL Advanced Haemosys software (version 3.26, Experimetria Ltd. and Logirex Software Laboratory, Budapest, Hungary). Coronary flow was calculated with timed collection of coronary effluent. Coronary flow was measured 1 min before the start of the experimental protocol, and at every 5th min of the protocol, and 1 min before and after switching perfusion solution during the protocol. At the end of the experiment, atria were removed from the hearts and ventricles were weighed. Coronary flow values are shown in ml/min/g. Hearts were equilibrated for 5–10 min before starting the experimental protocol.

### 2.3. Experimental protocol

In order to reduce repolarization reserve, and fully inhibit the main repolarizating current in guinea pig hearts, $I_{Ks}$ was selectively blocked by HMR-1556 at a concentration of 700 nM. The sensitivity of the model was assessed with two test drugs: one low concentration of the highly selective $I_{Kr}$ blocker dofetilide (10 nM) and one low concentration of the selective $I_{Kr}$ inhibitor cisapride (20 nM) were tested separately in two sets of experiments.

In the first set of experiments, four groups of hearts were compared: (1) control group of hearts perfused with dimethyl sulphoxide (DMSO), that is the solvent of dofetilide and HMR-1556 ('Control1' group; n = 10); (2) hearts perfused with HMR-1556 at 700 nM ('HMR1' group; n = 9); (3) hearts perfused with dofetilide at 10 nM ('DOF' group; n = 8); and (4) hearts co-perfused with dofetilide at 10 nM + HMR-1556 at 700 nM ('DOF + HMR' group; n = 9).

In the second set of experiments, hearts were perfused either with (1) DMSO, that is the solvent of cisapride and HMR-1556 ('Control2' group), or (2) HMR-1556 at 700 nM ('HMR2' group) or (3) cisapride at 20 nM ('CIS' group), or (4) cisapride at 20 nM + HMR-1556 at 700 nM ('CIS + HMR' group). Each group contained n = 6 hearts in the second set of experiments.

In the first set of experiments, the protocol started with a 20-min-long drug-free perfusion ('Drug-free' period). This was followed by the 20-min-long 'Drug' period, when the selective $I_{Ks}$ inhibitor HMR-1556, or the selective $I_{Kr}$ inhibitor dofetilide, or the combination of the two drugs (HMR-1556 + dofetide), or their solvent (DMSO) was added to the perfusate. Since the function of the $I_{Ks}$ is strongly influenced by the activity of the autonomic nervous system (Verrier & Antzelevitch, 2004), catecholamines (adrenaline at 25 nM + noradrenaline at 100 nM) were added to the buffer in the third period of the protocol in order to increase the function of $I_{Ks}$ and to mimic the presence of the sympathetic activity in denervated isolated hearts (Orosz et al., 2014). That is, the 'Drug' period was followed by

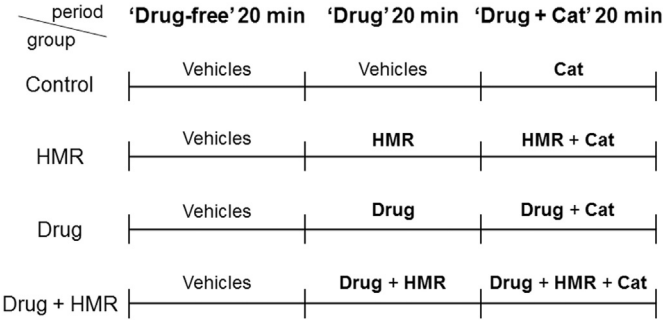


**Fig. 1.** Experimental protocol. Vehicles: vehicles are water acidified with ascorbic acid (the solvent of the catecholamines) and DMSO (the common solvent of dofetilide and HMR-1556); HMR: 700 nM HMR-1556; drug: 10 nM dofetilide in the first set of experiments or 20 nM cisapride in the second set of experiments; Cat, catecholamines (adrenaline 25 nM + noradrenaline 100 nM).