# Comparison of different proarrhythmia biomarkers in isolated rabbit hearts

Summary of PhD Thesis

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Szeged, 2015

## THE THESIS IS BASED ON THE FOLLOWING PAPERS

I. The role of the Na+/Ca2+ exchanger, I(Na) and I(CaL) in the genesis of dofetilide-induced torsades de pointes in isolated, AV-blocked rabbit hearts.
Farkas AS, Makra P, Csík N, **Orosz S**, Shattock MJ, Fülöp F, Forster T, Csanády M, Papp JG, Varró A, Farkas A.
Br J Pharmacol. 2009 Mar;156(6):920-32. doi: 10.1111/j.1476-5381.2008.00096.x. Epub 2009 Feb 16
**IF.: 5.067**

II. The assessment of efficacy of proarrhythmia biomarkers in isolated rabbit hearts with attenuated repolarization reserve.
**Orosz S**, Sarusi A, Csík N, Papp JG, Varró A, Farkas S, Forster T, Farkas AS, Farkas A.
J Cardiovasc Pharmacol. 2014 Sep;64(3):266-76. doi: 10.1097/FJC.0000000000000116.
**IF.: 2.383**

**Cumulative impact factor of papers directly related to the thesis: 7.45**

# 1 INTRODUCTION

## 1.1 Background of the study

The cardiovascular diseases accounted for more deaths than any other single cause of death in the United States since 1900. Pharmaceutical companies are really interested in the development of great number of antiarrhythmic drugs, since tachyarrhythmias are the most frequent causes of sudden cardiac death (SCD).

"Torsades de Pointes" (TdP) often associated with proarrhythmia by drugs that cause QT-prolongation. TdP is considered a life-threatening form of polymorphic ventricular tachycardia, which can be evoked by several cardiac or non-cardiac drugs. TdP has a characteristic pathophysiology and can manifest as acutely decreased pump function and haemodynamic instability, leading to syncope or via transformation to ventricular fibrillation, causing SCD.

## 1.2 Biomarkers in prediction of Torsades de Pointes

As the overall incidence of TdP is very low, there is a great need to find highly sensitive and specific surrogate biomarkers of TdP. To date, among many suggested biomarkers, still the ECG QT and heart rate corrected QT (QTc) intervals are the most accepted and the most frequently-used TdP surrogates. However, the predictive power of these parameters has been questioned.

In order to overcome this problem, we developed a method to allow ECG intervals to be measured during disorganized non-sinus rhythm before TdP occurrence. This allowed established beat-to-beat variability parameters to be derived irrespective of rhythm. To differentiate from published beat-to-beat variability parameters described by others, all of which are derived during stable rhythm (*sinus* beat-to-beat variability parameters), we coined the term *absolute* to describe the derived beat-to-beat variability parameters of the ECG intervals. *Absolute* beat-to-beat variability parameters of the ECG intervals accurately predicted drug-induced TdP and also the development of ischaemic VF, whereas equivalent variables measured in sinus rhythm failed to predict TdP and VF liability.

## 1.3 Aims of the study

Most pharmaceutical companies conduct cardiovascular safety studies before selection of candidate drugs for development. The *in vitro*, isolated, rabbit heart model relatively simple and does not require high amount of drug, we chose this model for our studies.

The primary objective of the first study was to investigate the role of Na+/Ca2+ exchanger (NCX) in the development of TdP in AV ablated isolated rabbit hearts. Although we focused in our study especially the antiarrhythmic effect of the inhibition of the L-type Ca2+ current ($I_{CaL}$) by verapamil and the inhibition of the $I_{Na}$ by lidocaine against dofetilide-induced TdP in isolated, Langendorff-perfused, AV-ablated rabbit hearts. Also, an attempt was made to determine whether the occurrence of dofetilide-induced TdP was related to the prolongation of the rate corrected QT (QTc) interval or the beat-to-beat variability of the QT interval in the applied model.

The aim of second study was to investigate which TdP biomarker (QTc, *sinus* or *absolute* beat-to-beat variability of the QT interval) can adequately indicate the increased risk of proarrhythmia in the setting of reduced repolarization reserve in an *in vitro*, spontaneous beating rabbit heart proarrhythmia model. Reduced repolarization reserve was achieved by concomitant pharmacological inhibition of $I_{Kr}$ and $I_{Ks}$.

## 2 MATERIALS AND METHODS

Female New Zealand white rabbits were used for both studies. The heart was removed and the aorta was hung on a Langendorff apparatus. The hearts were retrogradely perfused. The perfusion pressure was maintained constant at 80 mmHg. Electrocardiogram (ECG) was recorded, coronary flow was measured.

In AV ablated rabbit hearts an incision was made in the right atrium and the AV node was ablated using forceps. AV ablation was regarded successful when the P wave was dissociated from the QRS complex on real-time ECG recording.

### *2.1.1 Experimental protocol in isolated AV ablated rabbit heart study*

The experiments were design to examine whether it is possible to reduce dofetilide-induced TdP with other drugs. lidocaine and verapamil were chosen as test drugs as they could successfully reduce the incidence of drug-induced TdP. The experiments comprised three groups of hearts (see Table 2).

| Group | n | Pretreatment (20 min) | Treatment (30 min) |
|---|---|---|---|
| **Dofetilide 100 nM** | 8 | DMSO | DMSO+dofetilide |
| **Lidocaine 30 µM+Dofetilide 100 nM** | 8 | DMSO+lidocaine | DMSO+lidocaine+dofetilide |
| **Verapamil 750 nM+Dofetilide 100 nM** | 8 | DMSO+verapamil | DMSO+verapamil+dofetilide |

**Table 1.** The experimental protocol applied in isolated, Langendorff-perfused, AV-blocked rabbit hearts.

### *2.1.2 Experimental protocol in isolated, reduced repolarization reserve rabbit heart study*

In the first set of experiments three groups of hearts were compared (see Table 3).

| PERIOD / GROUP | Pretreatment (30 min) | Treatment (30 min) |
|---|---|---|
| Control A (n=6) | vehicle | vehicle |
| Dof 15 (n=8) | vehicle | dofetilide 15 nM |
| HMR + Dof 15 (n=6) | HMR | HMR + dofetilide 15 nM |

**Table 2.** Experimental protocol in the first set of experiments. 'Dof 15': group perfused with 15 nM dofetilide; 'HMR + Dof 15': group perfused with 460 nM HMR-1556 and 15 nM dofetilide. HMR: HMR-1556 at 460 nM, vehicle: DMSO (dimethyl sulphoxide), the common solvent of the dofetilide and HMR-1556

In the first set of experiments there were only few drug-induced arrhythmias, therefore we performed a second set of experiments, in which we intended to increase the incidence of arrhythmias. In the second set of experiments four groups (each contained 8 hearts) were compared (see Table 3).

| PERIOD / GROUP | Pretreatment (30 min) | Treatment (30 min) |
|---|---|---|
| Control B (n=8) | vehicles | vehicles |
| Cat control (n=8) | Cat. + vehicle | Cat. + vehicle |
| Cat + Dof 50 (n=8) | Cat. + vehicle | Cat. + dofetilide 50 nM |
| Cat + HMR + Dof 50 (n=8) | Cat. + HMR | Cat. + HMR + dofetilide 50 nM |

**Table 3.** Experimental protocol in the second set of experiments. 'Cat + Dof 50': group perfused with catecholamines and 50 nM dofetilide; 'Cat + HMR + Dof 50': group perfused with catecholamines, 460 nM HMR-1556 and 50 nM dofetilide. Cat.: catecholamines (epinephrine 25 nM + norepinephrine 100 nM). Vehicles in the 'Control B' group are water acidified with ascorbic acid (the solvent of the catecholamines) and DMSO (the common solvent of the dofetilide and HMR-1556). Vehicle in the other groups is only DMSO. For further details see Table 3.

In the first set of experiments we aimed to achieve a sufficient impairment of the repolarization without provoking a prominent arrhythmic activity. Thus, the selective $I_{Kr}$ blocker dofetilide was applied at a concentration of 15 nM, which inhibited approximately 50% of the $I_{Kr}$ $K^+$ current and led to QTc prolongation without significant arrhythmic activity.

In the second set of experiments we intended to increase the incidence of arrhythmias by further repolarization impairment. The concentration of dofetilide was increased to 50 nM in order to achieve complete inhibition of $I_{Kr}$. Since $I_{Ks}$ blockade does not significantly increased repolarization instability unless β-adrenergic stimulation is added in canines *in vivo*, catecholamines were added to the perfusion solution to mimic the sympathetic activity and to boost the function of $I_{Ks}$ (and the effect of $I_{Ks}$ inhibition) in the isolated rabbit hearts.

### 2.2 Analysis

ECG intervals (QT, QTc, RR, QRS) were measured at predetermined time points in both studies.

In both studies, to determine *sinus* and *absolute* beat-to-beat variability of the RR and QT intervals, all analyses were based on samples of 40 consecutive RR intervals at the predetermined time points.

Complete arrhythmia analysis and flow measurement were performed in both studies.

## 3 RESULTS

### 3.1 The in vitro, isolated, AV ablated rabbit heart model

Mechanical AV block significantly decreased the ventricular heart rate in all groups and caused a chaotic and *irregular* spontaneous ventricular rhythm, the average QT interval lengthened significantly due to the increased RR intervals. The measurement of ECG

variability parameters was only possible during arrhythmias in many experiments. The measured absolute QT and RR variability data also significantly increased and sensitize the hearts for the genesis of arrhythmias in the presence of proarrhythmic drugs.

*3.1.1 The RR and the QTc intervals*

The mean RR interval and the mean QTc interval did not significantly differ between the lidocaine, verapamil and the control groups before dofetilide perfusion, in the 'Pretreatment period'. When dofetilide was added to the perfusate during the 'Treatment period' verapamil further increased the dofetilide-induced QTc prolongation. However, lidocaine did not affect the mean QTc interval. verapamil on top of dofetilide perfusion significantly increased the mean RR interval.

*3.1.2 The absolute variability of the RR and QT intervals*

QT or RR variability parameters did not significantly differ between the lidocaine, verapamil and the control groups before dofetilide perfusion neither during 'Pretreatment period' nor during treatment period.

*3.1.3 TdP incidences*

In the experiments, dofetilide provoked TdP in the majority of the hearts (88%). Lidocaine significantly decreased the incidence of dofetilide-induced TdP (13%), while verapamil completely prevented the development of this arrhythmia (0%). Thus, both lidocaine and verapamil could significantly decrease the incidence of TdP in this model.

*3.1.4 Coronary flow*

Perfusion with verapamil significantly increased the coronary flow. Lidocaine did not influence the coronary flow.

**3.2 The in vitro, isolated, reduced repolarization reserve rabbit heart model**

*3.2.1 QTc interval and heart rate in sinus rhythm*

First set of experiments

QTc did not differ between the groups in the pretreatment period. In the treatment period, administration of dofetilide for 10 min significantly prolonged the QTc interval, however QTc did not differentiate between the 'Dof 15' and the 'HMR + Dof 15' groups. There was no significant difference in the heart rate between the groups during the whole experiment.

Second set of experiments

QTc did not differ between the groups at baseline and in the pretreatment period, before dofetilide perfusion. In the treatment period, administration of dofetilide for 10 min significantly widened the QTc interval, however QTc did not differentiate between the 'Cat + Dof 50' and the 'Cat + HMR + Dof 50' groups dofetilide-induced arrhythmias prevented further measurements of the biomarker at the subsequent time points.

*3.2.2 Arrhythmia incidences and onset time of arrhythmias*

First set of experiments

Dofetilide induced TdP in one heart (13%) in the 'Dof 15' group; the drug did not evoke ventricular fibrillation. There were no significant differences in the onset times of arrhythmias between the groups.

Second set of experiments

As expected, catecholamines increased arrhythmic activity. In the pretreatment period, catecholamine perfusion evoked mostly VPBs, the incidences of other types of arrhythmias (BG, salvo, VT) were low, there were no significant differences in the incidences of arrhythmias between the groups. TdP and VF did not occur in this period.

In the treatment period, addition of dofetilide on top of HMR-1556 and catecholamines increased the incidence of BG, salvo, VT and VF, but the effect was not significant as compared with control. TdP occurred in one heart in both dofetilide-perfused groups. Co-perfusion of dofetilide, HMR-1556 and catecholamines significantly increased the incidence of conduction blocks as an indirect sign of markedly prolonged repolarization. There were no significant differences in the onset times of arrhythmias between the groups.

*3.2.3 Temporal inhomogeneity of the repolarization and the cycle length*

First set of experiments

The *sinus* STV QT and the *sinus* LTV QT parameters did not differ between the groups at baseline and during the pretreatment period. Importantly, both *sinus* STV QT and *sinus* LTV QT increased significantly in the 'HMR + Dof 15' group as compared with the values in the 'Dof 15' and the 'Control A' groups. There was no significant difference in any of the variability parameters of the RR interval at any time points between the groups.

Second set of experiments

*The sinus variability parameters of the ECG intervals*

Dofetilide co-administered with HMR-1556 for 5 min tended to increase the *sinus* STV QT and LTV QT in the 'Cat + HMR + Dof 50' group as compared with the values of the 'Cat + Dof 50' and the control groups.

*The absolute beat-to-beat variability of the ECG intervals*

None of the *absolute* variability parameters of the RR and QT intervals differed between the groups at baseline and during the pretreatment period. In the treatment period, co-perfusion of dofetilide with HMR-1556 increased the *absolute* variability parameters of the QT and RR intervals.

# 4 Discussion

## 4.1 The in vitro, isolated, AV ablated rabbit heart model

### *4.1.1 AV block induced a chaotic rhythm and an electrical instability*

AV block led to a chaotic idioventricular rhythm under drug-free conditions which was accompanied by an elevated beat-to-beat variability of the RR and QT intervals. The inhibition of the $Na^+$ channels by lidocaine as well as the block of the L-type $Ca^{2+}$ channels by verapamil significantly antagonized the genesis of dofetilide-induced Torsades de Pointes. However, verapamil further increased the dofetilide-induced QTc prolongation and neither verapamil nor lidocaine reduced the dofetilide-induced increase in the beat-to-beat variability of the QT interval. Thus, neither QTc prolongation nor an increase in the beat-to-beat variability of the QT interval is a sufficient prerequisite of TdP genesis in rabbit hearts.

### *4.1.2 The antitorsadogenic effect of verapamil and lidocaine*

The $I_{CaL}$ channel blocker verapamil significantly prolonged the QT interval in the presence of dofetilde and prevented the development of TdP. Verapamil has never been reported to cause TdP and indeed has been proposed as a therapy for long QT–related arrhythmias, so verapamil is considered as a relatively safe antiarrhythmic drug. However, verapamil can inhibit $I_{Kr}$ in the same concentration range as quinidine and amiodarone. This may explain why the drug prolonged further the QT interval in our study, when the repolarization reserve was very small as a result of dofetilide perfusion. Lidocaine suppressed the $I_{Kr}$ blocker almokalant-induced dispersion of repolarization and the development of EADs in rabbit Purkinje fibres *in vitro*. Thus, the antiarrhythmic effect of verapamil and lidocaine in

our experiments may also be related their direct effect on the development of EAD and/or dispersion of the repolarization.

## 4.2 The in vitro, isolated, reduced repolarization reserve rabbit heart model

### *4.2.1 QTc failed to predict increased proarrhythmia risk in reduced repolarization reserve*

QTc failed to predict the known increased risk of TdP in the setting of reduced repolarization reserve. The QT and heart rate corrected QT (QTc) interval prolongation is the solely predictor of TdP that is unequivocally received by the authorities, although, their sensitivity and specificity has been strongly questioned. Dofetilide alone and co-perfused with the $I_{Ks}$ blocker HMR-1556 caused an equivalent degree of QTc prolongation, thus the extent of the QTc prolongation did not differ between the presence and absence of the $I_{Ks}$ inhibition. These results emphasize that QTc interval is not a suitable biomarker to identify increased TdP liability in case of reduced repolarization reserve.

### *4.2.2 Sinus beat-to-beat variability of the repolarization is a better predictor of TdP in reduced repolarization reserve, but arrhythmic activity limits its use*

It has been suggested that temporal dispersion of repolarization could predict proarrhythmic events. In the first set of experiments in the present investigation the measurement of the beat-to-beat variability of the QT intervals was possible in sinus rhythm as the arrhythmic activity was low during concomitant pharmacological inhibition of $I_{Ks}$ and $I_{Kr}$ in isolated rabbit hearts. In the group perfused with dofetilide together with HMR-1556 the beat-to-beat variability parameters of QT interval were significantly increased as compared with those in the control group, and more importantly, compared with those in the group perfused only with dofetilide. Accordingly, STV and LTV QT could differentiate the combined ion-channel block from the solely $I_{Kr}$ block identifying the increased TdP liability during decreased repolarization reserve.

However, in the second set of experiments, catecholamines and elevated concentration of dofetilide increased the arrhythmic activity, which precluded the measurement of the *sinus* beat-to-beat variability parameters of the QT intervals, thus these parameters failed to indicate the increased proarrhythmic liability of the drugs during reduced repolarization reserve. These results show that s*inus* beat-to-beat variability parameters of the repolarization (*sinus* STV QT and LTV QT) are better predictors of TdP than QTc in reduced repolarization reserve, although the application of these parameters is limited when arrhythmia occurs.

### *4.2.3 Absolute beat-to-beat variability of the repolarization is the best proarrhythmia predictor in reduced repolarization reserve*

In the presence of reduced repolarization reserve ectopic beats can render the APD unstable for many beats and thus produce large beat-to-beat irregularity. However, increased variability of repolarization may remain latent until the heart is disturbed by an ectopic beat. Since TdP rarely occurs spontaneously without preceding arrhythmias, however, the number of arrhythmic beats was not a precise predictor of drug-induced TdP, new TdP biomarkers that can be measured irrespective of the rhythm even during arrhythmias are needed. The *sinus* beat-to-beat variability of the repolarization can predict TdP development, but its predictive power is not consistent especially when frequent arrhythmias preclude its measurement. However, the newly developed *absolute* beat-to-beat variability parameters of the ECG intervals seem to be more reliable surrogate biomarkers of TdP. Indeed, in the present study the *absolute* beat-to-beat variability parameters of the repolarization indicated the increased proarrhythmic liability of dofetilide in the setting of decreased repolarization reserve.

### *4.2.4 The efficacy of the applied proarrhythmia biomarkers in TdP forecast*

The applied absolute variability parameter allow the analysis of ECG segments during arrhythmia, therefore, the beat-to-beat ECG analysis is not only restricted to sinus or stable, regular rhythm. The beat-to-beat variability parameters tended to be more sensitive than QTc, or simple arrhythmia analysis alone in the TdP prediction. However, the specificity of variability parameters is still merit further assessment.

## 5 Acknowledgements

I would like to thank Professor András Varró, Head of the Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Szeged, for his support.

I would also like to thank Dr Attila Farkas, my tutor and supervisor, who directed my research work for his invaluable help during my PhD studies.

I wish to acknowledge Dr András Farkas for his support and help during my PhD studies.

I greatly acknowledge to Dr Sándor Farkas. He greatly helped me improve my scientific thinking, reasoning and presentation skills.

I am thankful to Tímea Kajtár for her skillful assistance.

And I would like to thank my family for their support.