Combined Effects of Acquired LQT Syndrome by Dofetilide and Reduced Repolarization Reserve on Human Ventricular Action Potential: A Simulation Study

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Abstract

The aim of this study was to investigate interactions between acquired long QT syndrome by dofetilide and reduced repolarization reserve in simulated human ventricular cells using prolongation of APD and triangulation of action potential. A Markovian model of hERG capable of reproducing a wild type as well an acquired long QT syndrome by dofetilide was developed and was included into a ventricular cell model for action potential simulation. Dofetilide concentrations used were 7.5, 30 and 100 nM. The endocardial and mid-myocardial cells were paced with different basic cycle lengths (750, 1000 and 1250 ms). The reduced repolarization reserve was reproduced decreasing the IKs current by 50 and 25%. The results pointed out that triangulation offers an additional more sensitive and accurate indicator of the proarrhythmic potential of a drug than APD prolongation alone.

1. Introduction

The heart is a rhythmic electromechanical pump, the functioning of which depends on action potential generation and propagation, followed by relaxation and a period of refractoriness until the next impulse is generated [1]. Myocardial action potentials reflect the sequential activation and inactivation of inward (Na^+ and Ca^{2+}) and outward (K^+) current carrying ion channels. In different regions of the heart, action potential waveforms are distinct, owing to differences in Na^+, Ca^{2+}, and K^+ channel expression, and these differences contribute to the normal, unidirectional propagation of activity and to the generation of normal cardiac rhythms, see Figure 1.

One of the major regulatory concerns on the development of new drugs is acquired long QT syndrome. The QT interval is defined as the time interval between the onset of the QRS complex and the end of the T wave, and therefore, includes both the ventricular depolarization and repolarization intervals. Because the QT interval reflects the duration of individual action potentials in cardiac myocytes, prolongation of the action potential duration (APD) will result in a prolonged QT interval.

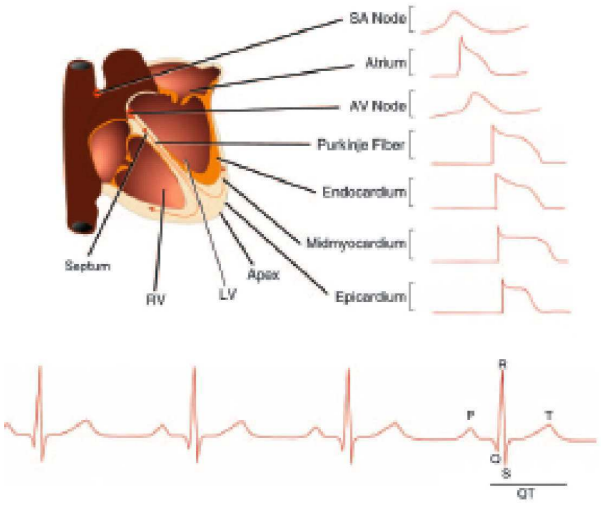

Figure 1. Propagation of action potential in the heart.

Cardiac APD is controlled by a fine balance between inward and outward currents in the plateau and repolarization phases[2]. Since outward K^+ currents, especially the delayed rectifier repolarizing current, IK (which is the sum of two kinetically and pharmacologically distinct types of K^+ currents: a rapid, IKr, and a slow, IKs, component), play an important role during plateau – repolarization and in determining the configuration of the action potential, small changes in conductance can significally alter the effective refractory period, hence the action potential duration. In particular, most of the QT prolonging drugs have been shown to inhibit the K^+ channels encoded by the human ether-a-go-go-related gene (hERG), at the basis of the rapid component of IK, named IKr. Blockade of the hERG K^+

channel is, therefore, the most important mechanism through which QT prolonging drugs increase cardiac potential duration.

The APD starts with the upstroke (phase 0) and ends with terminal fast repolarization (phase 3), see Figure 2. Because phases 0 and 1 are usually relatively short, the APD is primarily the sum of phases 2 and 3. Consequently, the APD can be lengthened by prolongation of phase 2, phase 3, or both. The channels carrying the current during phase 2 and phase 3 are substantially different. Indeed during the plateau, the small declining inward currents flow primarily through slowly inactivating sodium channels and L-type calcium channels, whereas the progressively increasing outward currents flow through potassium channels (to a large extent IKs, because IKr is inactivated primarily at more positive potentials in the plateau). During phase 3 repolarization, open inward channels promptly close by deactivation, but IKr and IK1 potassium channels open by removing rectification, whereas IKs slowly deactivates. Consequently, drugs can prolong phase 2 by increasing inward currents (sodium/calcium) or by reducing outward currents (IKs). Drugs can slow phase 3 repolarization by closing IKr or IK1 channels. Promiscuous drugs can prolong both phases 2 and 3 by interacting with ion channels of both groups.

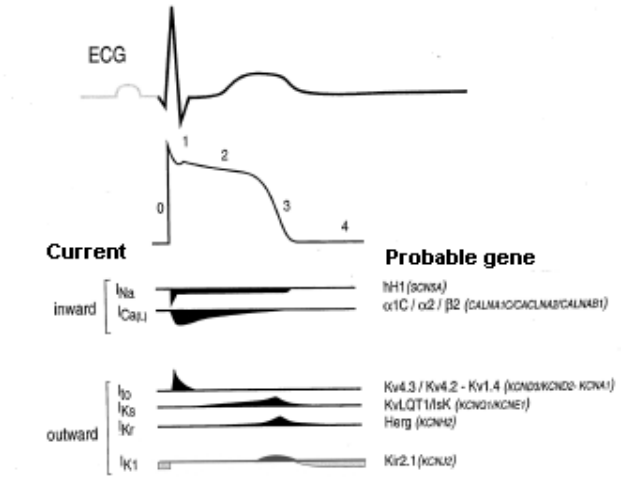

Figure 2. Ventricular action potential and main inward and outward currents.

Repolarization reserve is the intrinsic aspect of a cell to maintain normal repolarization despite impairments in the ability of a cell to repolarize. One way of framing the concept of reduced repolarization reserve is with defective slow component of delayed rectifier potassium current (IKs). In normal situation, this current contributes to the repolarization process only to a minimal extent, but has a vital rol in the repolarization reserve [3]. IKs can be decreased not exclusively by ion channel mutation but it can be down-regulated due to diseases such as heart failure, diabetes and cardiac hypertrophy.

The aim of this study was to investigate interactions between acquired long QT syndrome by dofetilide and reduced repolarization reserve in simulated human ventricular cells using prolongation of APD and triangulation of action potential.

2. Methods

In order to simulate the effect of acquired long QT syndrome by dofetilide on cardiac behaviour, a continuous-time Markov model of hERG was used [4] and the interaction of dofetilide with IKr/hERG dynamics was modelled. The Markov model state diagram is illustrated in Figure 3, which shows the interaction of dofetilide in accordance with experimental studies that have shown drug-receptor interaction in open and in inactivated states [5].

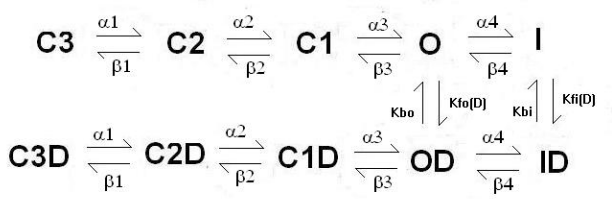

Figure 3. Markov model state diagram with dofetilide interaction.

Dofetilide is a class III antiarrhythmic drug for treatment of patients with persistent atrial fibrillation and flutter. It is a specific blocker of IKr/hERG. Dofetilide blocks IKr in all myocardial tissues with high potency. Block is voltage dependent and most prominent at depolarized potentials.The dofetilide concentrations used were 7.5, 30 and 100 nM (IC_{50}= 7.5 nM).

The developed Markovian model was incorporated into the Ten Tusscher ventricular cell model [6] in order to simulate the action potential and analyze the blockade of IKr/hERG by dofetilide and reduced repolarization reserve. The endocardial and mid-myocardial cells were paced with different basic cycle length (750, 1000 and 1250 ms).

The concept of triangulation has been developed as an additional in vitro biomarker for proarrhythmia [7]. It quantitatively measures the slowing of repolarization in monophasic action potentials. The triangulation of action potential was calculated as the difference between APD_{90} (APD at 90%) and APD_{40} (APD at 40%). The reduced repolarization reserve was reproduced decreasing the IKs current by 50 and 25%.

3. Results

Dofetilide produces a prolongation of APD both in M and endocardial cells of myocardium. Figure 4 shows the prolongation of APD with different concentrations of dofetilide in M cells with a BCL of 1000 ms. Moreover, dofetilide was found to produce a marked concentration dependent triangulation of action potential such that 7.5, 30 and 100 nM produced an increase of 11, 24 and 27 ms in mid-myocardial cells, whereas in endocardial cells the increase was 6, 10 and 11 ms respectively.

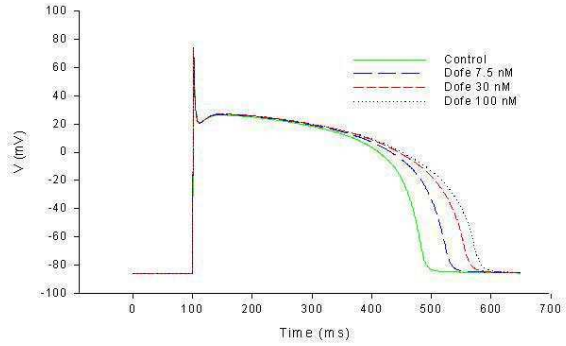

Figure 4. Action potential and prolongation of APD with different concentrations of dofetilide in M cells with a BCL of 1000 ms.

Reduced repolarization reserve alone produced a slight triangulation increase from 2 to 3 ms in midmyocardial cells whilst in endocardial cells was from 3 to 5 ms. Reduced repolarization reserve prolongs the APD, which is illustrated in Figure 5.

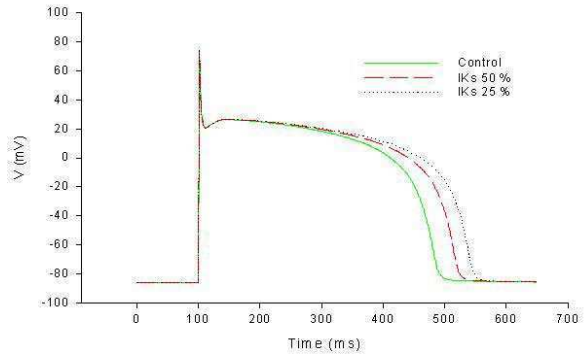

Figure 5. Action potential and prolongation of APD with reduced repolarization reserve of 50 and 25% in M cells with a BCL of 1000 ms.

The combination of drug effects (dofetilide concentration of 100 nM) with reduced repolarization reserve (25% of IKs) produced a maximal APD prolongation of 235 ms in midmyocardial cells with a BCL of 1000 ms, see Figure 6, and a triangulation increase of 43 ms, whereas in endocardial cells the APD prolongation was 150 ms and the triangulation increase was 25 ms.

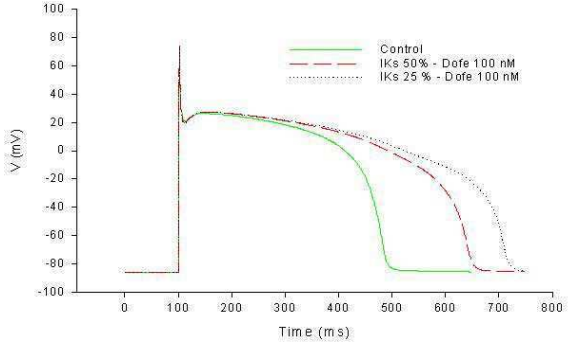

Figure 6. Action potential and APD prolongation with combined effects in M cells with a BCL of 1000 ms.

Table 1 and Table 2 show the effects of increasing concentrations of dofetilide and reduced repolarization reserve on APD, APD_{90} and on triangulation in M cells and endocardial cells respectively with a BCL of 1000 ms. At different basic cycle lenghts of 750, 1000 and 1250 ms, the triangulation of action potential was not found to be significantly modified.

Table 1. Effects of increasing concentrations of dofetilide and reduced repolarization reserve on APD, APD_{90} and on triangulation in M cells with a BCL of 1000 ms.

	Dofe (nM)	TRIAN (ms)	APD_{90} (ms)	APD (ms)
Control	0	36	386	401
	7.5	47	428	452
	30	60	461	486
	100	64	478	503
IKs50%	0	38	420	439
	7.5	50	473	493
	30	62	519	540
	100	71	546	566
IKs25%	0	39	444	461
	7.5	52	510	529
	30	68	573	592
	100	79	617	636

Figure 7 shows the APD on increasing concentrations of dofetilide with and without reduced repolarization reserve. Although APD was significant modified from 401 to 636 ms, its increases were uniforme, almost with the same slope.

Table 2. Effects of increasing concentrations of dofetilide and reduced repolarization reserve on APD, APD_{90} and on triangulation in endocardial cells with a BCL of 1000 ms.

	Dofe (nM)	TRIAN (ms)	APD_{90} (ms)	APD (ms)
Control	0	37	297	337
	7.5	43	312	354
	30	47	322	365
	100	48	326	370
IKs50%	0	40	351	378
	7.5	47	373	401
	30	52	387	416
	100	55	394	424
IKs25%	0	42	401	422
	7.5	51	432	453
	30	58	454	476
	100	62	465	487

One of the mechanisms for the increased proarrhythmogenecity is not due to a simple delay of repolarizaction but an increase in the slope of the final repolarization. Figure 8 shows triangulation on increasing concentrations of dofetilide with and without reduced repolarization reserve, in which was demonstrated that IKs becomes an important factor controlling the slope of final repolarization (triangulation) in the context of IKr block.

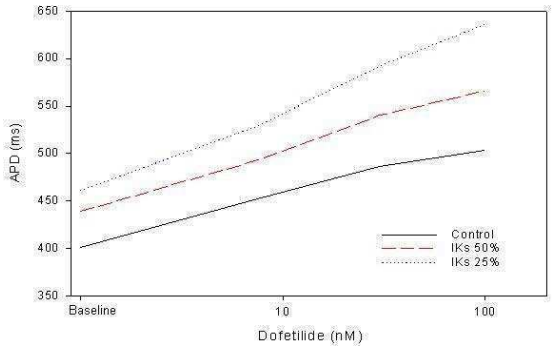

Figure 7. APD on increasing concentrations of dofetilide with and without reduced repolarization reserve.

In normal situations, IKs contributes to the repolarization process only to a minimal extent, but has a vital role in the repolarization reserve. Excesive APD prolongation induced by IKr blockade constitutes a good substrate for time dependent IKs activation which finally increases its participation to the ventricular repolarization.

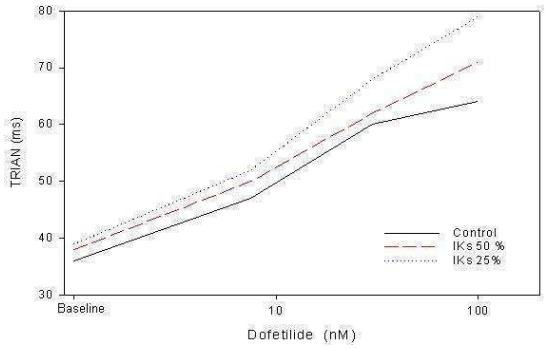

Figure 8. Triangulation on increasing concentrations of dofetilide with and without reduced repolarization reserve.

4. Discussion and conclusions

This study had shown the interactions between acquired long QT syndrome by dofetilide and reduced repolarization reserve in simulated human ventricular cells and pointed out that triangulation offers an additional more sensitive and accurate indicator of the proarrhythmic potential of a drug than APD prolongation alone.

References

[1] Nerbonne JM, Kass RS. Molecular physiology of cardiac repolarization. Physiol Rev, 2005; 85:1205-1253.

[2] Tristani-Firouzi M, Chen J, Mitcheson JS, Sanguinetti MC. Molecular biology of K+ channels and their role in cardiac arrhythmias. Am J Med, 2001;110: 50-59.

[3] Roden DM. Taking the ¨ idio¨ out of ¨idiosyncratic¨ predicting torsades de pointes. Pacing Clin Electrophysiol, 1998; 21:1029-1034.

[4] Fink M, Noble D. Contributions of HERG K^+ current to repolarization of the human ventricular action potential. Phil Trans A Math Phys Eng Sci, 2006; 364:1207-1222.

[5] Weerapura M, Hebert TE, Nattel S. Dofetilide block involves interactions with open and inactivated states of Herg channels. Pflugers Archiv. 2002; 443: 520-531.

[6] Ten Tusscher K, Panfilov A. Alternans and spiral breakup in human ventricular tissue model. Am J Physiol Heart Circ. 2006; 291: H1088-H1100.

[7] Hondeghem LM., Carlsson L, Duker G. Instability and triangulation of the action potential predict serious proarrhythmia, but action potential prolongation is antiarrhythmic. Circulation, 2001; 103: 2004-2013.

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