Original article

A new biomarker – index of Cardiac Electrophysiological Balance (iCEB) – plays an important role in drug-induced cardiac arrhythmias: beyond QT-prolongation and Torsades de Pointes (TdPs)

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TdPs: Torsades de Pointes

Abstract

Introduction: In the present study, we investigated whether a new biomarker – index of cardiac electrophysiological balance (iCEB = QT/QRS) – could predict drug-induced cardiac arrhythmias (CAs), including ventricular tachycardia/ventricular fibrillation (VT/VF) and Torsades de Pointes (TdPs). Methods: The rabbit left ventricular arterially-perfused-wedge was used to investigate whether the simple iCEB measured from the ECG is reflective of the more difficult measurement of λ (effective refractory period-conduction velocity) for predicting CAs induced by a number of drugs. Results: Dofetilide concentration-dependently increased iCEB and λ, predicting potential risk of drug-induced incidence of early afterdepolarizations (EADs) starting at 0.01 μM, Digoxin (1 and 5 μM), encainide (5 and 20 μM) and propranolol (10 and 100 μM) markedly reduced both iCEB and λ, predicting their ability to induce non-TdP-like VT/VF. At 10 μM, both NS1643 and levomilast significantly decreased λ and iCEB, which was preceded with presence of non-TdP-like VT/VF. Isoprenaline (0.05 to 0.5 μM) significantly reduced both λ and iCEB, which was associated with a high incidence of non-TdP-like VT/VF in most preparations. Other biomarkers (i.e. transmural dispersion of T-wave and instability of the QT interval) predicted only dofetilide-induced long QT and EADs, but did not predict drug-induced risk of non-TdP-like VT/VF. Discussion: Our data from 7 reference drugs of known pro-arrhythmic effects suggests that 1) this non-invasive iCEB predicts potential risk of drug-induced CAs beyond long QT and TdP; 2) iCEB is more useful than the current biomarkers (i.e. transmural dispersion and instability) in predicting potential risks for drug-induced non-TdP-like VT/VF.

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1. Introduction

Drug-induced QT prolongation and the appearance of Torsade de Pointes (TdP) are recognized as a potential risk associated with the use of a broad range of cardiovascular and non-cardiovascular drugs (Cubeddu, 2003; Pugsley, Authier, & Curtis, 2008; Shah, 2004). Current and past established regulatory guidelines (CPMP/986/96 points to consider document, 1997 and ICH S7B, 2005) recommend the conduct of preclinical studies both in vivo and in vitro to detect drug-induced QT-interval prolongation and arrhythmogenic potential. The preclinical guidance was followed more recently by the ICH E14 recommendation for conducting thorough QT (TQT) investigations in early clinical trials (2005). Drug-induced long QT and its risk for TdP are now well known, because in recent years, extensive progress has been made in studying the congenital long QT syndromes (Roden, 2004), and also different screening strategies for detecting drug-induced TdP have been evaluated following the release of CPMP/986/96 in 1997.

However, cardiovascular safety in general is still the major cause of drug attrition in early drug development and of drug withdrawal from the market (Laverty et al., 2011). In some cases, the identification of cardiovascular risks is neither detected in earlier clinical trials nor deemed to be biologically or clinically significant until the new medicines are given to large patient populations for a long period on the market (Psaty & Furberg, 2007; Laverty et al., 2011; Redfern et al., 2010).
These cardiovascular risks can lead to limitations in the use of the medicine, or to drug withdrawal, and can be caused not only by drug-induced long QT and TdP, but also by other factors (Heist & Ruskin, 2010; Shah, 2010). With the exception of patients with genetic hERG defects, little is known about the implications of QT-shortening caused by drugs (Lu, Hermans, & Gallacher, 2012; Shah, 2010) or slowing of cardiac conduction time (widening of QRS) (Gintant, Gallacher, & Pugsley, 2011; Harmer, Valentijn, & Pollard, 2011; Lu et al., 2010; Madias, 2008). The Cardiac Arrhythmia Suppression Trial (CAST, 1989) showed that flecainide and encainide [Class IC antiarrhythmic, sodium current (INa) blocking agents] were associated with an increased incidence of sudden cardiac death in post-infarction patients (Sellers & DiMarco, 1984; Echt et al., 1991). Some published papers show that there are large numbers of drugs which block INa current and could have the propensity to induce cardiac arrhythmias (Gintant et al., 2011; Harmer et al., 2011; Lu et al., 2010). Moreover, some drugs that shorten the QT-interval could also have the potential to induce non-TdP-like VT/VF (VT: ventricular tachycardia; VF: ventricular fibrillation) (Lu et al., 2006). Since it is difficult to capture QT shortening in the setting of the associated fatal arrhythmia (VF), due to lack of 24 h holter monitoring data, and to capture a slowing down of conduction associated with non-TdP-like VT/VF, details concerning drug-induced short QT interval and slowing of conduction are limited. Indeed, the present ICH S7B guideline (Food and Drug Administration, HHS, 2005) does not specifically address the potential for drug-induced slowing of conduction (QRS widening), acquired short QT, and their potential for the development of fatal arrhythmias. Therefore, the currently-applied biomarkers (e.g. instability of QT and transmural dispersion of repolarization-T wave: TDR) may not be adequate to detect all types of drug-induced cardiac arrhythmias.

Therefore, in the present study, we would like to introduce a new and non-invasive biomarker: “the index of Cardiac-Electrophysiological Balance” (iCEB), which is the ratio of QT/QRS of the ECG. We speculate that 1) increases/decreases in QT or JT-interval are proportional to those in effective refractory period (ERP), and 2) changes in QRS are similar to those in conduction velocity (CV). Therefore, the iCEB (the ratio of QT/QRS) derived from the ECG, should be the equivalent of the classic λ (ERP/CV), and significant changes in iCEB may reflect an imbalance in cardiac electrophysiology, and therefore predict cardiac arrhythmias. Therefore, iCEB could be used as a new biomarker to detect drug-induced broad cardiac arrhythmias including TdP and non-TdP-like VT/VF (Fig. 1).

![Fig. 1. Balance and imbalance of the depolarization (QRS duration) and repolarization (QT interval) of the cardiac electrophysiology. Schematic changes in the index of Cardiac-Electrophysiological Balance (iCEB): slight increases/decrease (↑ or ↓) in iCEB by increase/decrease in QT interval or QRS duration could be no-arrhythmic or even antiarrhythmic (green zone) (balance between the depolarization and repolarization of cardiac electrophysiology) while large changes (↑↑ or ↓↓) in iCEB may be proarrhythmic (imbalance of cardiac electrophysiology) (dark magenta color). TdPs: Torsades de Pointes, VT: ventricular tachycardia, VF: ventricular fibrillation.](image)
2.2. Electrophysiological recordings and ERP measurement from the rabbit ventricular wedge preparation

A transmural pseudo-ECG signal was recorded using extracellular silver/silver chloride electrodes placed in the Tyrode’s solution bathing the preparation 1.0 to 1.5 cm from the epicardial and endocardial surface of the left ventricle (not whole the heart).

From the ECG, we measured transmural dispersion of repolarization which is approximately equal to the interval between the peak and the end of the T wave ($|T_{peak} - T_{end}|$), and $rT_P-Te$ ($|T_P-Te| = |T_P-T_E|/(QT+100)$ (Liu et al., 2006; Yan & Antzelevitch, 1999). The intervals of the ECG are similar to those in the intact heart but without P wave. 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 crossed the isoelectric line. The JT interval is calculated as the QT interval minus QRS duration (QT interval − QRS duration). The conduction velocity $CV$ (cm/s) was calculated by dividing the longest distance from the edge of the preparation to the pacing electrode by the QRS duration. The programmed stimulation at 2-fold the diastolic threshold from the endocardium was used to determine the effective refractory period (ERP). The cardiac wavelength ($\lambda$) was calculated by ERP (ms) × conduction velocity (CV: cm/s) (Giroir & Rosenbaum, 2001). All parameters were recorded at a BCL of 500 ms and of 2000 ms. iCEB was calculated by both the ratio of QT/QRS or JT/QRS, measured from the ECG recordings. The QRS duration, QT, JT and Tp–Te intervals were measured manually from 5 consecutive beats within the last minute of the recording period and the values were then averaged. In order to estimate conduction velocity, the size of each preparation was measured.

The beat-to-beat instability (variability) of the QT interval was defined according to the method used in our recent studies (Lu, Vlaminckx, Van de Water, & Gallacher, 2006; Van der Linde et al., 2005), and similar to the beat-to-beat variability of repolarization in dogs (Thomsen et al., 2004) and in rabbits (Jacobson et al., 2011). In brief, data are taken from 20 to 30 consecutive QT intervals and plotted in a Poincaré plot. The distance to the x coordinate represents the length of the plot and reflects the long-term instability, and the distance to the y coordinate is the width of the plot and reflects the short-term instability. After calculation of these distances for all data points, the median value is calculated for the instability parameters of the total tracing of consecutive 20 to 30 beats. In the present study, we reported the short-term beat-to-beat instability as beat-to-beat instability in the present study (Van der Linde et al., 2005). Tdp score was also calculated in each group (Liu et al., 2006).

Ventricular tachycardia (VT) was defined as four or more consecutive ventricular premature beats, ventricular fibrillation (VF) was defined as a ventricular tachycarrhythmia without identifiable ECG, and in-excitability was defined as the phenomenon where the preparation could not follow the electrical stimulation.

2.3. Study protocol

Stock solutions of the reference drugs were prepared in DMSO. Each compound was tested at 4 different concentrations in 6 wedge preparations. After one-hour equilibration, during which the preparation achieved electrical stability (Yan et al., 1998, 2001; Liu et al., 2006), one of the following 8 treatments was applied for 30 min per concentration (n = 6 per group).

The preparation was paced at three basic cycle lengths (BCLs) of 500 ms, 1000 ms and 2000 ms. A brief period (30 to 60 s) of faster pacing at a BCL of 500 ms or less was introduced between BCLs of 1000 ms and 2000 ms using bipolar silver electrodes.

2.4. Compounds and their selection

Encainide, propoxyphene, NS1643, isoprenaline, digoxin and levocromakalim were obtained from Sigma-Aldrich® (Belgium). Dofetilide was synthesized by TOSLab Ltd. (Ekaterinburg, Russia). Dimethyl sulfoxide (DMSO, final maximal bath concentration 0.1%) was used as solvent control. The concentrations of the compounds, selected for these studies, were based on published IC$_{50}$ values for their respective target blockade or activation and on their reported effects in proarrhythmic in vitro models (Bentzen et al., 2010; Harmer et al., 2011; Kennedy, Seifen, Aker, & Brody, 1986; Lenz & Hilleman, 2000; Lu et al., 2008; Rhodes et al., 2012; Ulens, Daenens, & Tygat, 1999; Wu, Carlsson, Liu, Kowey, & Yan, 2005).

2.5. Data analysis

All values are expressed as mean and standard error of the mean (S.E.M.). Statistically significant differences between solvent and compound were calculated based on their changes from baseline with the Wilcoxon–Mann–Whitney test. Two-tailed probabilities of less than 0.05 were considered to indicate statistically significant differences.

3. Results

3.1. Dofetilide (an I$_{Kr}$ blocker, mimicking drug-induced Long QT2)

Dofetilide ($n = 6$) concentration-dependently significantly increased both iCEB (ratio of QT/QRS or JT/QRS) (+79% to 168%) and cardiac wavelength ($\lambda = ERP \times CV$) (+96 to 194% from baseline at 0.01 μM, 0.1 μM, 0.3 μM and 1 μM, respectively; p<0.05 versus solvent, n = 6) (Table 1, and Fig. 2).

Dofetilide elicited early afterdepolarizations (EADs) in 2, 6, 6 and 6 out of the 6 preparations at 0.01, 0.1, 0.3 and 1 μM, respectively (versus 0 out of the 6 preparations with solvent; p<0.05 at 0.1, 0.3 and 1 μM, respectively) (see Fig. 2 for an example of dofetilide-induced EADs at 0.01 μM in an isolated arterially-perfused rabbit left ventricular wedge).

Dofetilide also concentration-dependently increased transmural dispersion (expressed as $rT_P-Te$) by 28% to 53% from baseline (versus 0% to + 1% from baseline with solvent; p<0.05) and concentration-dependently increased the instability of the QT interval starting at 0.01 μM (p<0.05 only at 1 μM) (Table 2). As expected, dofetilide also concentration-dependently prolonged the QT interval (+79% to +146% from baseline versus 0% to +4% from baseline with solvent; p<0.05), and the JT interval (+90% to 169% from baseline versus 0% to +4% from baseline with solvent; p<0.05) without significantly changing QRS duration (Table 4). Furthermore, dofetilide significantly increased the Tdp score starting at 0.01 μM (7 versus 0 with solvent; p<0.05). The Tdp score reached a maximum of 10 at 0.1, 0.3 and 1 μM, respectively (7 to 10 of baseline versus 0 with solvent; p<0.05).

3.2. Digoxin (an activator of Na$^+$/K$^+$ ATPase pump)

Digoxin at 0.01 and 0.1 μM did not significantly or physiologically relevantly change either iCEB or iCEB. However, at 1 and 5 μM, digoxin significantly decreased both iCEB and $\lambda$ (to the same degree as $\lambda$ (~7% to −65% of baseline versus +1% to +3% of baseline with solvent; p<0.05)) (Table 1 and Fig. 3: upper part).

Digoxin elicited non-Tdp-like ventricular tachycardia (VT) in 4 out of the 6 preparations at 5 μM (versus 0 out of the 6 preparations; p<0.05), and ventricular fibrillation (VF) in 1 and 6 out of the 6 preparations at 1 and 5 μM, respectively (versus 0 out of the 6 preparations with solvent; p<0.05 at 5 μM) (see an example in Fig. 3).

Digoxin at 0.01, 0.1 and 1 μM did not significantly produce relevant change $rT_P-Te$ (Table 2). At 5 μM, $rT_P-Te$ could not be measured due to extra beats and changes in the morphology of the T wave.
Digoxin did not significantly change the instability of the QT interval at the lower doses tested. Instability could not be measured at the two highest doses in some experiments (in 2 and 3 out of 6 preparations at 1 and 5 μM, respectively), because of frequent premature beats.

Digoxin at 1 and 5 μM significantly shortened the QT interval (−11% and −43% from baseline versus +2% and +4% from baseline with solvent; *p < 0.05) and the JT interval (−12% and −48% from baseline versus +2% and +4% from baseline with solvent; *p < 0.05), without significantly changing QRS duration (Table 4).

### Table 1

Effects of 7 reference compounds on the cardiac wavelength (λ) and index of Cardiac Electrophysiological Balance (iCEB) in the rabbit left ventricular arterially-perfused-wedge.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose 1 (μM)</th>
<th>Dose 2 (μM)</th>
<th>Dose 3 (μM)</th>
<th>Dose 4 (μM)</th>
<th>(actual unit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ (ERPxCV) Solvent</td>
<td>10.7 ± 1</td>
<td>2 ± 1</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
<td>(Change of baseline)</td>
</tr>
<tr>
<td>Dofetilide</td>
<td>10 ± 1</td>
<td>96 ± 21*</td>
<td>160 ± 32*</td>
<td>194 ± 29*</td>
<td>182 ± 24*</td>
</tr>
<tr>
<td>Digoxin</td>
<td>10.3 ± 1</td>
<td>2 ± 1</td>
<td>3 ± 2</td>
<td>−7 ± 2*</td>
<td>−65 ± 2*</td>
</tr>
<tr>
<td>Encainide</td>
<td>11 ± 1</td>
<td>1 ± 2</td>
<td>−12 ± 2*</td>
<td>−40 ± 5</td>
<td></td>
</tr>
<tr>
<td>NS1643</td>
<td>10.8 ± 0.3</td>
<td>3 ± 1</td>
<td>4 ± 2</td>
<td>0 ± 1</td>
<td>−18 ± 3*</td>
</tr>
<tr>
<td>Propoxyphene</td>
<td>10.4 ± 0.2</td>
<td>4 ± 1</td>
<td>4 ± 2</td>
<td>−8 ± 3*</td>
<td>−45 ± 3*</td>
</tr>
<tr>
<td>Levocromakalim</td>
<td>9.8 ± 1</td>
<td>1 ± 1</td>
<td>−1 ± 1</td>
<td>−2 ± 1</td>
<td>−69 ± 4*</td>
</tr>
<tr>
<td>Isopropylaline</td>
<td>10 ± 1</td>
<td>−9 ± 4</td>
<td>−18 ± 3*</td>
<td>−17 ± 2*</td>
<td>−22 ± 9*</td>
</tr>
</tbody>
</table>

Data are Mean ± S.E.M. of n=6 per group. ERP: effective refractory period; CV: conduction velocity. λ was calculated by ERPxCV (in cm); iCEB was calculated by QT/QRS or JT/QRS ratio.*: P<0.05 versus solvent group and indicated in bold. Doses of the reference compounds are listed in the respective figure.

### Table 2

Comparison the effects of 7 reference compounds on iCEB with those on the transmural dispersion (rTp-Te) of T wave and instability of QT-interval (in ms) in the rabbit left ventricular arterially-perfused wedge.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose 1 (μM)</th>
<th>Dose 2 (μM)</th>
<th>Dose 3 (μM)</th>
<th>Dose 4 (μM)</th>
<th>(Change of baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>iCEB (QT/QRS) Solvent</td>
<td>7.8 ± 1</td>
<td>2 ± 1</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
<td>0</td>
</tr>
<tr>
<td>Dofetilide</td>
<td>8.4 ± 1</td>
<td>79 ± 19*</td>
<td>119 ± 22*</td>
<td>146 ± 21*</td>
<td>145 ± 23*</td>
</tr>
<tr>
<td>Digoxin</td>
<td>8.8 ± 0.4</td>
<td>3 ± 2</td>
<td>4 ± 2</td>
<td>−11 ± 3*</td>
<td>−45 ± 5*</td>
</tr>
<tr>
<td>Encainide</td>
<td>8.2 ± 1</td>
<td>3 ± 1</td>
<td>3 ± 2</td>
<td>−11 ± 3*</td>
<td>−41 ± 4*</td>
</tr>
<tr>
<td>NS1643</td>
<td>8.7 ± 1</td>
<td>2 ± 1</td>
<td>3 ± 2</td>
<td>−1 ± 1</td>
<td>−20 ± 4*</td>
</tr>
<tr>
<td>Propoxyphene</td>
<td>8.3 ± 0.5</td>
<td>2 ± 1</td>
<td>−1 ± 4</td>
<td>−14 ± 4*</td>
<td>−54 ± 3*</td>
</tr>
<tr>
<td>Levocromakalim</td>
<td>8.7 ± 0.4</td>
<td>2 ± 1</td>
<td>1 ± 2</td>
<td>2 ± 4</td>
<td>−61 ± 2*</td>
</tr>
<tr>
<td>Isopropylaline</td>
<td>8.1 ± 1</td>
<td>−9 ± 4</td>
<td>−21 ± 3*</td>
<td>−19 ± 9</td>
<td>−31 ± 4*</td>
</tr>
</tbody>
</table>

Data are Mean ± S.E.M. of n=6 per group. *: values could not be measured due to arhythmias in some of 6 preparations.

### 3.3. Isoprenaline (a β₁- and β₂-adrenoceptor agonist)

Isoprenaline at 0.05 to 0.5 μM significantly reduced λ (−18% to −22% from baseline versus +2% to +3% from baseline with solvent; *p < 0.05) and iCEB to a similar degree (−21% to −31% from baseline versus +1% to +3% from baseline with solvent; *p < 0.05) at 0.05 and 5 μM. Isoprenaline elicited non-TdP-like VT in 4, 6 and 6 out of 6 preparations.
at 0.05 μM, 0.1 μM and 0.5 μM, respectively (versus 0 out of the 6 preparations with solvent; p < 0.05) (Fig. 3; lower part).

Isoprenaline did not significantly change the instability of the QT interval at doses up to 0.5 μM, and did not increase, but tended to decrease rTp-Te (Table 2). In the presence of isoprenaline at 0.01 μM, the instability of the QT interval could not be determined in about 50% of the preparations due to extra beats, and also Tpeak and the Tdp score could not be measured correctly.

Isoprenaline shortened the QT interval (−9%, −21%, −20% and −31% from baseline at 0.01 μM, 0.05 μM, 0.1 μM and 0.5 μM, respectively, versus ≤ +4% from baseline with solvent; p < 0.05, except for 0.1 μM). Isoprenaline did not significantly change QRS duration (Table 4) and QRS-rate dependency (data not shown).

Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>iCEB</th>
<th>QRS-duration</th>
<th>ERP</th>
<th>QT-interval instability</th>
<th>rTp-Te</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dofetilide</td>
<td>↑</td>
<td>±</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>EAD</td>
</tr>
<tr>
<td>Digoxin</td>
<td>↓</td>
<td>±</td>
<td>↓</td>
<td>±</td>
<td>±</td>
<td>VT/VF</td>
</tr>
<tr>
<td>Encainide</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>±</td>
<td>±</td>
<td>VT/VF</td>
</tr>
<tr>
<td>Ns1643</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>±</td>
<td>±</td>
<td>VT/VF</td>
</tr>
<tr>
<td>Propoxyphene</td>
<td>↓</td>
<td>±</td>
<td>↓</td>
<td>±</td>
<td>±</td>
<td>VT/VF</td>
</tr>
<tr>
<td>Levromakalim</td>
<td>↓</td>
<td>±</td>
<td>↑</td>
<td>±</td>
<td>±</td>
<td>VT/VF</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>↓</td>
<td>±</td>
<td>↓</td>
<td>±</td>
<td>±</td>
<td>VT/VF</td>
</tr>
</tbody>
</table>

iCEB: index of Cardiac Electrophysiological Balance, ERP: effective refractory period; EAD: early afterdepolarization; VT: ventricular tachycardia; VF: ventricular fibrillation. ↑: significant increase; ↓: significant decrease; ±: no significant change. Data are only taken at the dose with incidence or the highest dose tested.

Table 4

<table>
<thead>
<tr>
<th>Compound</th>
<th>Baseline</th>
<th>Dose 1</th>
<th>Dose 2</th>
<th>Dose 3</th>
<th>Dose 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>iCEB/QR/QRS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Solvent</td>
<td>7.8±0.5</td>
<td>7.8±0.5</td>
<td>7.9±0.6</td>
<td>8.0±0.5</td>
<td>8.1±0.5</td>
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<tr>
<td>Dofetilide</td>
<td>8.4±0.7</td>
<td>14.9±1.9</td>
<td>18.1±2</td>
<td>20.2±2.2</td>
<td>20±2.2</td>
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<tr>
<td>Digoxin</td>
<td>8.8±0.4</td>
<td>9.0±0.5</td>
<td>9.1±0.5</td>
<td>7.8±0.7</td>
<td>4.8±0.4*</td>
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<tr>
<td>Encainide</td>
<td>8.1±0.5</td>
<td>8.4±0.6</td>
<td>8.4±0.6</td>
<td>7.3±0.1</td>
<td>4.8±0.3*</td>
</tr>
<tr>
<td>Ns1643</td>
<td>8.7±0.4</td>
<td>8.8±0.4</td>
<td>8.9±0.4</td>
<td>8.6±0.4</td>
<td>7.8±0.5*</td>
</tr>
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<td>Propoxyphene</td>
<td>8.3±0.5</td>
<td>8.4±0.6</td>
<td>8.1±0.6</td>
<td>7.1±0.4</td>
<td>3.8±0.3*</td>
</tr>
<tr>
<td>Levromakalim</td>
<td>8.7±0.4</td>
<td>8.9±0.4*</td>
<td>8.7±0.4</td>
<td>8.8±0.5</td>
<td>3.4±0.2*</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>8.1±0.6</td>
<td>7.3±0.4</td>
<td>6.4±0.5*</td>
<td>6.2±0.5</td>
<td>5.4±0.4*</td>
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Table 5

<table>
<thead>
<tr>
<th>Compound</th>
<th>Baseline</th>
<th>Dose 1</th>
<th>Dose 2</th>
<th>Dose 3</th>
<th>Dose 4</th>
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<td>QRS-duration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Solvent</td>
<td>37±2</td>
<td>37±2</td>
<td>37±2</td>
<td>37±2</td>
<td>37±2</td>
</tr>
<tr>
<td>Dofetilide</td>
<td>36±2</td>
<td>36±2</td>
<td>36±2</td>
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<td>36±2</td>
</tr>
<tr>
<td>Digoxin</td>
<td>35±1</td>
<td>35±1</td>
<td>35±1</td>
<td>35±1</td>
<td>36±1</td>
</tr>
<tr>
<td>Encainide</td>
<td>37±2</td>
<td>38±2</td>
<td>39±2</td>
<td>48±4</td>
<td>73±9</td>
</tr>
<tr>
<td>Ns1643</td>
<td>35±2</td>
<td>35±2</td>
<td>35±2</td>
<td>36±2</td>
<td>39±3*</td>
</tr>
<tr>
<td>Propoxyphene</td>
<td>37±2</td>
<td>37±2</td>
<td>40±2</td>
<td>47±3</td>
<td>95±8*</td>
</tr>
<tr>
<td>Levromakalim</td>
<td>34±1</td>
<td>34±1</td>
<td>34±1</td>
<td>34±1</td>
<td>34±1</td>
</tr>
<tr>
<td>Isoprenaline</td>
<td>38±1</td>
<td>38±1</td>
<td>37±1</td>
<td>37±1</td>
<td>37±1</td>
</tr>
</tbody>
</table>

ERP

| Solvent | 248±13 | 248±14 | 252±14 | 254±13 | 257±13 |
| Dofetilide | 243±17 | 466±41 | 618±66 | 695±48 | 669±41* |
| Digoxin | 248±16 | 252±19 | 253±22 | 230±20 | 89±9 |
| Encainide | 261±12 | 271±14 | 278±16 | 297±15 | 302±15* |
| Ns1643 | 250±7 | 255±9 | 258±10 | 258±10 | 225±10* |
| Propoxyphene | 247±6 | 255±6 | 261±7 | 283±6 | 343±5 |
| Levromakalim | 232±8 | 232±9 | 231±9 | 230±8 | 71±6 |
| Isoprenaline | 238±9 | 216±12 | 194±19 | 193±19 | 182±12 |

Data are Mean±S.E.M. of n=6 per group. iCEB: index of Cardiac Electrophysiological Balance, ERP: effective refractory period. *: P < 0.05 versus solvent group in bold. Doses of the reference compounds are listed in the respective figure.

3.4. Encainide (a class lc antiarrhythmic agent, an I enhanced blocker)

Encainide at 0.5 and 1 μM did not significantly change both λ and iCEB. However, at 5 and 20 μM, encainide significantly reduced λ (ERPcv: −12% and −40% from baseline). Encainide significantly reduced iCEB (expressed as ratio of QT/QRS or JT/QRS) in a similar manner (−11% to −47% from baseline) (Table 1 and Fig. 5). At 20 μM, encainide elicited non-TdP-like VT in 4 out of the 6 preparations (versus 0 out of the 6 preparations with solvent; p < 0.05) (Fig. 4; lower part).

Encainide slightly but significantly prolonged the QT interval (+5%, +8%, +14% and +12% from baseline at 0.5 μM, 1 μM, 5 μM and 20 μM, respectively, versus ≤ +4% from baseline with solvent; p < 0.05 except for at 20 μM). Encainide dose-dependently increased QRS duration (+3%, +5%, +29% and +96% from baseline, at 0.5 μM, 1 μM, 5 μM and 20 μM, respectively, versus ≤ +1% from baseline with solvent; p < 0.05 except for at 0.5 μM) (Table 4), and QRS-rate-dependency (+5%, +18% and +61% from baseline at 1 μM, 5 μM and 20 μM, respectively, versus 0% of baseline with solvent; p < 0.05 except for at 0.5 μM), without significantly changing Tdp score (−1 to +1 of baseline).

Furthermore, encainide at 0.5 μM, 1 μM and 5 μM did not significantly change rTp-Te. At 20 μM, encainide did not increase, but significantly decreased rTp-Te (−33% from baseline versus 0% from baseline with solvent p < 0.05) (Table 2). Encainide did not significantly change the instability of the QT interval (Table 2).

3.5. Propoxyphene (a weak opioid agonist that presents with non-TdP-like arrhythmias associated with overdose in man)

Propoxyphene at 0.1 and 1 μM did not significantly change λ and iCEB. However, at 10 and 100 μM, propoxyphene significantly reduced λ (ERPcv: −8% and −45% from baseline) and iCEB (expressed as a ratio of QT/QRS or JT/QRS) to a similar extent (−14% to −61% from baseline) (Table 1 and Fig. 4; upper part). At 100 μM, propoxyphene elicited non-TdP-like VT in 4 out of the 6 preparations (versus 0 out of the 6 preparations with solvent; p < 0.05) (Fig. 4) and in-excitatibity (the preparation had no response to the electrical stimulation) in 3 out of the 6 preparations (versus 0 out of 6 preparations with solvent; p < 0.05).

Propoxyphene did not increase, but significantly reduced rTp-Te (−11%, −25% and −30% from baseline at 1 μM, 10 μM and 100 μM, respectively, versus 0% from baseline with solvent) (Table 2). Propoxyphene at the doses tested, did not significantly change the instability of the QT interval (Table 2). Propoxyphene at 100 μM significantly prolonged the QT interval (+17% from baseline versus +4% from baseline with solvent; p < 0.05), but did not significantly change the duration of the JT interval. Propoxyphene significantly
increased QRS duration (+25% and +153% from baseline at 10 μM and 100 μM versus ≤+1% from baseline with solvent; *: p<0.05) (Table 4) and QRS-rate-dependency (+3%, +20% and +84% from baseline at 1 μM, 10 μM and 100 μM, respectively, versus 0% of baseline with solvent; *: p<0.05) without notable changes in the TdP score (0 to −1 of baseline).

Fig. 3. Effects of digoxin and isoprenaline on cardiac λ (ERPxCV) and iCEB (QT/QRS ratio) in the isolated rabbit left ventricular arterially-perfused-wedge (left side of the figure). Values were expressed as mean±S.E.M., and as % changes from baseline, *: p<0.05 versus solvent. On the right side of the figure: an example of original ECG tracings recorded from a rabbit left ventricular wedge preparation at control period (Control) and in the presence of digoxin at 5 μM or isoprenaline at 0.05 μM. The results indicate that iCEB was equal to λ in predicting digoxin- and isoprenaline-induced non-TdP-like ventricular tachycardia (VT) and ventricular fibrillation (VF).

Fig. 4. Effects of propoxyphene and encainide on cardiac λ (ERPxCV) and iCEB (QT/QRS ratio) in the isolated rabbit left ventricular arterially-perfused-wedge (left side of the figure). Values were expressed as mean±S.E.M., and as % changes from baseline, *: p<0.05 versus solvent. On the right side of the figure: examples of original ECG tracings recorded from a rabbit left ventricular wedge preparation at control period (Control) and in the presence of propoxyphene at 100 μM and encainide at 20 μM with VT. The results indicate that iCEB was equal to λ in predicting encainide- and propoxyphene-induced non-TdP-like ventricular tachycardia (VT).
3.6. Levocromakalim (an IKATP channel opener, mimicking short QTs)

Levocromakalim at 10 μM largely reduced both λ (expressed as ERPxCV: −69% from baseline versus +3% from baseline with solvent; \( p<0.05 \)). Similarly, levocromakalim at 10 μM also markedly reduced iCEB (expressed as QT/QRS or JT/QRS: −61% or −69% from baseline, respectively, versus +3% or +4% from baseline with solvent; \( p<0.05 \)) (Table 1 and Fig. 5). Levocromakalim elicited non-TdP-like VF in 4 out of the 6 preparations (versus 0 out of the 6 preparations with solvent; \( p<0.05 \)) (see an example in Fig. 5).

Levocromakalim tended to increase rTp-Te at 10 μM (+27% from baseline versus 0% from baseline with solvent; \( p>0.05 \)), but did not significantly change instability of the QT interval at the doses tested (Table 2). As expected, levocromakalim at 10 μM largely shortened the QT interval (−60% from baseline versus +4% from baseline with solvent; \( p<0.05 \)) and the JT interval (−68% from baseline versus +4% from baseline with solvent; \( p<0.05 \)) without significantly changing QRS-duration and QRS-rate dependency. The TdP score was slightly reduced at 10 μM (−1 of baseline versus 0 of baseline with solvent; \( p<0.05 \)).

3.7. NS1643 (an IkR activator mimicking genetic short QT syndrome)

NS1643 at 10 μM significantly reduced λ (expressed as ERPxCV: −18% from baseline versus +3% from baseline with solvent; \( p<0.05 \)). Similarly, NS1643 at 10 μM also significantly reduced iCEB (expressed as QT/QRS or JT/QRS: −20% or −22% from baseline, respectively, versus +3% or +4% from baseline with solvent; \( p<0.05 \)) (Table 1 and Fig. 5). NS1643 elicited non-TdP-like VT/VF in 1 out of the 6 preparations (versus 0 out of the 6 preparations with solvent; \( p<0.05 \)) (see an example in Fig. 5).

NS1643 at 10 μM did not increase but decreased rTp-Te (−20% from baseline versus 0% from baseline with solvent, \( p<0.05 \)). As expected, NS1643 at 10 μM significantly shortened the QT interval (−11% from baseline versus +4% from baseline with solvent; \( p<0.05 \)) and the JT interval (−14% from baseline versus +4% from baseline with solvent; \( p<0.05 \)) without significantly changing QRS duration except for a slight increase at the highest dose (Table 4) and QRS-rate dependency, and with a slight reduction in TdP score at 10 μM (−1 of baseline versus 0 with solvent; \( p<0.05 \)). NS1463 at doses tested up to 10 μM did not significantly change the instability of the QT interval (Table 2).

In summary, significant increases or reductions in iCEB (QT/QRS ratio) are associated with its potential risk of induction of long QT/EADs or non-TdP types of VT/VF (Table 3).

4. Discussion

Our data with 7 cardio-active reference compounds tested in the isolated rabbit ventricular wedge preparation shows that the new non-invasive biomarker, index of Cardiac-Electrophysiological Balance (iCEB: the ratio of QT/QRS), is preceded with not only drug-induced long QT and TdP, but also to drug-induced slowing of conduction, QT shortening and their related non-TdP-like VT/VF. iCEB, measured from the electrocardiogram (ECG), could serve as an important, new and non-invasive biomarker for potential risks of drug-induced cardiac arrhythmias beyond LQTS and TdP, since currently used biomarkers i.e. transmural dispersion (rTp-Te) and instability of the QT interval are limited to the prediction of drug-induced long QT and TdP.

4.1. Can the index of Cardiac-Electrophysiological Balance (iCEB) be used as a more easily measured substitute to the measurement of λ for detecting potential risks of drug-induced cardiac arrhythmias beyond TdP?

In the present study, our data from 7 reference compounds indicate that iCEB (ratio of QT/QRS or JT/QRS, taken directly from ECG recordings) changed parallel with the classic cardiac λ (ERPxCV) (Girouard & Rosenbaum, 2001) (Table 1). Drugs that only slightly increase or decrease iCEB may be safe, while drugs markedly increasing or decreasing this parameter could potentially be pro-arrhythmic (Fig. 1). λ has been used as a parameter to study ventricular reentrant tachycardia and atrial fibrillation (Aidonidis et al., 2009; Robert et al., 2010).
However, the classical cardiac $\lambda$ is calculated based on the invasive measurement of ERP by using extra electrical stimulation (s) to the heart in a cardiac electrophysiological pacing laboratory in man. Although $\lambda$ is also used as a biomarker in animal models, the invasiveness of the ERP measurement may limit the use of this biomarker as a screening tool/biomarker. In addition, as summarized in Table 1: significant drug-induced changes in $\lambda$ (ERPxCV) are similar to those in iCEB, and both biomarkers are useful to predict potential risks for drug-induced cardiac arrhythmias. However, iCEB is a simple parameter, directly measured from ECG recordings without invasive ERP measurement, and may have advantages over the classical $\lambda$ measurement, which is based on the invasive measurement of ERP.

However, if a drug (i.e. with a multiple ion-current blocking effects) significantly changes both the duration of the QT interval and QRS duration to a similar degree, but it does not significantly change the iCEB, whether this kind of drugs resulting in proarrhythmic or anti-arrhythmic are unknown in the present study and are beyond the scope of the present manuscript.

4.2. The new non-invasive biomarker—iCEB plays an important role in drug-induced cardiac arrhythmias beyond TdP

The ventricular waveforms on the body ECG are generated by both depolarization and repolarization of the ventricular myocytes’ action potentials, and present themselves as the QRS complex (depolarization) followed by the T wave (repolarization) (Yan, Lankipalli, Burke, Musco, & Kowey, 2003). In physiological conditions there is a dynamic balance between depolarization and repolarization. Slight deviations in the balance i.e. within depolarization and repolarization reserve, are harmless or even can be anti-arrhythmic. For example, lidocaine which is a safe antiarrhythmic drug in men, only slightly increases QRS duration. Nicorandil (an IK$^+$,ATP channel agonist) which slightly shortens the QT interval is also considered as a safe drug. Raloxifene is an oral selective estrogen receptor modulator (SERM) that has estrogenic actions on bone and anti-estrogenic actions on the uterus and breast. Raloxifene is clinically used without drug-induced long QT/TdPs. However, raloxifene has potent HERG and IKs blocking activities in vitro (Liu et al., 2010). All these 3 compounds: lidocaine ($n=6$ up to 100 μM), raloxifene ($n=6$ up to 10 μM) and nicorandil ($n=6$ up to 10 μM) have little effects on iCEB (changes <8%) and do not elicit cardiac arrhythmias in this model (our unpublished data). We suggest that further studies are needed to determine the threshold for a clinically relevant change in iCEB. Moreover, additional studies are also needed to establish the role for iCEB in safety risk assessment, either as a stand-alone parameter or in combination with other biomarkers, especially for drugs with multiple ion-current blocking activities such as azimilide and quinidine. In addition, similar to significant changes of all other biomarkers such as instability, transmural dispersion, QT-interval or QRS duration etc., significant changes in iCEB are not always directly linked to the incidence of arrhythmias for each individual experiment. Further studies with large sample size are wanted to further investigate if iCEB can predict whether certain subjects will respond with cardiac arrhythmias or not.

On the other hand, significant imbalances (either increases or decreases) between the depolarization and repolarization of the heart e.g. in pathological conditions (heart failure, inherited LQTs or Brugada syndrome etc.) or induced by different types of cardiac and non-cardiac drugs as we have seen in the present study, may be associated with arrhythmic conditions (Fig. 1). Patients with heart failure and Brugada syndrome prolonged QRS-duration thereby decreasing iCEB (QT/QRS) and were associated with increased incidence of sudden cardiac death ( Kashani & Barold, 2005; Otkubo et al., 2011). Patients with inherited LQTs significantly prolonged QT-interval thereby significantly increasing iCEB and are associated with incidence for TdPs. Large changes in the depolarization or repolarization of the action potential significantly change iCEB and may result in cardiac arrhythmic conditions.

Indeed, in the present study, dofetilide (a potent IKs blocker) induced long QT and high incidences of EADs (risk of TdP) (Lenz & Hilleman, 2000). This was associated with significant concentration-dependent increases in iCEB. These results indicate that iCEB predicted potential risks of drug-induced long QT and TdP risk. On the other hand, the induction of non-TdP-types of VT/VF was associated with significant and marked decreases in iCEB with drugs slowing conduction (propoxyphene and encainide) and shortening QT (NS1643, digoxin, isoprenaline and levcromakalim). The large decreases in iCEB may therefore be a prerequisite substrate for the initiation of non-TdP-types of VT/VF.

Propoxyphene is a synthetic opioid pain reliever and the FDA recommended its withdrawal from the market due to its risk of causing cardiac arrhythmias (not LQT and TdP related), heart failure and hypotension in 2010. Toxic blood concentrations range between 3 and 180 μmol/l (Cravey, Shaw, & Nakamura, 1974; Ulens et al., 1999).

Encainide was also shown to have a proarrhythmic potential in clinical trials [Cardiac Arrhythmia Suppression Trial (CAST)] (The CAST Investigators, 1989), and in experimental models (Nattel, 1998). In the CAST, IKs-current blockers such as encainide (Class IC) were associated with an increased incidence of sudden cardiac death in post-myocardial infarct patients (The CAST Investigators, 1989). Our results indicate that both propoxyphene (100 μM) and encainide (20 μM) markedly slowed conduction and thereby decreased iCEB, which was associated with the induction of non-TdP types of VT/VF (Fig. 5).

High toxic doses of digoxin (an activator of Na$^+$/K$^+$ ATPase pump) (Kennedy et al., 1986) NS1643 (an IKs activator, mimicking genetic short QT syndrome) (Lu et al., 2010), isoprenaline (a β1- and β2-adrenoreceptor agonist, mimicking catecholaminergic polymorphic VT) (Balasubramanian, Grace, Saumure, Vandenberg, & Huang, 2003) and of levcromakalim (an IKATPase opener), all largely reduced iCEB. Significant reductions in iCEB may be therefore associated with the risk for non-TdP types of VT/VF. The mechanisms by which digitalis over-dose or toxicity promotes the development of cardiac arrhythmias including fatal non-TdP types of VT/VF are still not completely clear. In the present study, decreases in iCEB preceded the induction of non-TdP types of VT/VF. It is already known that isoprenaline at high doses, induces cardiac arrhythmias (including delayed afterdepolarizations, triggered activities and non-TdP types of VT/VF) via β-adrenergic overly stimulation of IKs (L) which increases the concentration of Ca$^{2+}$. The Ca$^{2+}$-overload maybe caused by Ca$^{2+}$ release from the sarcoplasmatic reticulum (SR) via ryanodine 2 receptors (R,R) during diastole, and therefore provokes non-TdP-like VT/VF (Nam, Burashnikov, & Antzelevitch, 2005; Watanabe & Knollmann, 2011; Song, Wu, Shroyack, & Belardinelli, 2002). However, there are no clear biomarkers to predict digitalis and isoprenaline-induced arrhythmias. Our data show that iCEB can serve as a new biomarker to predict their potential risk of non-TdP like VT/VF.

Slowing of conduction (increasing QRS duration) was associated with sudden cardiac death in both man and in experimental models (Antzelevitch, 2007; Yan & Antzelevitch 1999; Lu et al., 2010; Lu, Hermans, & Gallagher, 2012; Nattel, 1998). Drugs which slow conduction, thereby decreasing iCEB, and associated with non-TdP-like VT/VF could be interpreted as “bad” IKs blocking drugs like encainide (Class IC) and propoxyphene (Lu et al., 2010, Lu, Hermans & Gallagher 2012). Indeed, changes in conduction time, ERP or both, promote re-entrant arrhythmias (Aidonidis et al., 2009; Robert et al., 1996, 1999; Lu et al., 2010; Lu, Hermans & Gallagher 2012). Drugs that increase $\lambda$ tend to increase the risk for TdPs, whereas agents that decrease the $\lambda$ tend to increase the risk for non-TdP VT/VF (Aidonidis et al., 2009; Hondeghem, Dujardin, Hoffmann, Dumotier, & De clerck, 2011; Robert et al., 1996, 1999). In 1913, Mines first suggested the role of $\lambda$ in the mechanism of reentry: reentrant excitation was only possible
if the $\lambda$ of the propagating impulse was shorter than the reentrant path length, i.e. that there is an excitable gap within the reentrant circuit (Mines, 1913). Later on, other studies also suggested that decreases in $\lambda$ by shortening the QT interval are related to the initiation and maintenance of reentry, which lead to reentrant VT or fibrillation (Robert et al., 1996; 1999; Aidonidis et al., 2009; Giroud et al., 1996). Robert et al. reported that levocamakalim-induced reentry VT in isolated rabbit hearts was associated with a shortening in $\lambda$ due to a direct decrease in ERP without changes in CV (Robert et al., 1999). Aidonidis et al. (2009) reported that bimakalim (another IK$_{ATP}$ channel opener) shortened ERP thereby decreased $\lambda$ and induced reentrant non-TdP-like VT/VF in chronic infarct anesthetized pigs. In the clinic, genetic short QT syndromes, characterized by abnormally short QT intervals (<300 ms), are linked to atrial fibrillation, syncope and sudden death due to non-TdP-like VF (Gaita et al., 2003; Giustetto et al., 2011). Therefore, a large decrease in iCEB is associated with drug-induced non-TdP-like VT/VF, while a marked increase in iCEB is associated with drug-induced TdPs.

4.3. Does iCEB have advantages over established biomarkers such as transmural dispersion (Tp-Te, rTp-Te) and instability of the QT interval?

QT interval prolongation is associated with Tdp, but QT prolongation per se is a poor biomarker of the risk of TdP actually occurring. Therefore, more predictable bio-markers for Tdp, including transmural dispersion of the T wave (Tp-Te, rTp-Te) and instability/variability of the QT interval, have been developed in recent years. Increases in transmural dispersion (rTp-Te) of the T wave and instability of the QT interval have been well established as associated with drug-induced long QT syndrome, and these two biomarkers are associated with drug-induced Tdp (Jacobson et al., 2011; Liu et al., 2006; Lu et al., 2006; Thomsen et al., 2004; Van der Linde et al., 2005; Yan & Antzelevitch, 1999). Indeed, in the present study, our results with dofetilide (an iK$_{r}$ blocker) showed that all 3 biomarkers including iCEB, rTp-Te and instability of the QT-interval predict the compound's ability to cause QT prolongation and are associated with a risk of producing Tdp. However, in the present study, drug-induced non-TdP-like VT/VF is generally not associated with increases in transmural dispersion or in instability of the QT-interval. Only iCEB appeared to be a good biomarker for this type of arrhythmia, as a decrease in iCEB is indicative of a risk for drug-induced non-TdP-like VT/VF (Tables 2 and 3).

With respect to the concept that increases in transmural dispersion may be a general parameter for arrhythmogenesis of LQT/TdP or of short QT (Anttonen et al., 2008; Extramiana & Antzelevitch, 2004; Antzelevitch, 2007; Antzelevitch et al., 2007; Yan & Antzelevitch, 1999), in the present study in the rabbit wedge preparation, we could not exclude its potential role in drug-induced non-TdP-like VT/VF. Encaïnine (20 μM), NS1643 (10 μM) and propoxyphene (at the highest 3 doses) significantly reduced transmural dispersion of T wave, in contrast to increases as reported in literature (Anttonen et al., 2008; Extramiana & Antzelevitch, 2004; Antzelevitch, 2007; Antzelevitch et al., 2007; Yan & Antzelevitch, 1999). The reason for this discrepancy is unknown. In cases of drug-induced changes in morphology of the T wave (which occurs in the presence of Na$^{+}$ channel blockers or QT-shortening drugs), it is difficult to accurately measure the dispersion (Tp-Te of QT) from ECG recordings. Furthermore, it is also difficult to continuously measure the QT interval when there are extra beats in the rabbit wedge model, hampering a correct calculation of instability.

Indeed, in isolated wedge preparations, compounds like encaïnine and propoxyphene markedly widened the QRS complex, and altered the morphology of the T wave (flattened, negative or bi-phasic) — Tp-Te can’t be accurately measured in these circumstances. In case of administration of a high dose of digoxin or isoprorenaline, instability was not always measurable, because there were incidences of extra beats or VT/VF, and it was therefore impossible to analyze 20 to 30 consecutive QT intervals.

5. Conclusion

This non-invasive, simple and new biomarker iCEB represents the balance between the depolarization (changes in QRS duration) and repolarization (changes in the QT interval) of the cardiac action potential, while transmural dispersion of the T wave and instability of the QT interval only cover alterations in repolarization (changes in the QT interval) of the action potential. Therefore, the new biomarker iCEB may have advantages over the current biomarkers in identifying the potential risk for cardiac arrhythmias, especially for its potential to discriminate between long-QT related arrhythmias and non-TdP-like VT/VF.

The present study in rabbit left ventricular arterially-perfused wedge suggests that the iCEB may constitute a potentially important and non-invasive biomarker in predicting a higher predisposition to different types of drug-induced cardiac arrhythmias beyond LQT and Tdp. However, this left ventricular arterially-perfused wedge preparation is an in vitro model under a condition of pacing. The importance of this biomarker in predicting cardiac arrhythmias in other models both in vitro and in vivo still needs to be established. Furthermore, the value of iCEB as a tool to predict potential arrhythmias in humans, especially in diseased conditions, needs to be evaluated in future studies.

In conclusion, our data from 7 compounds with known cardiac effects suggest that: 1) this non-invasive iCEB is qualitatively similar to $\lambda$, gives an indication towards a higher likelihood for drug-induced CAs beyond long QT and Tdp; 2) iCEB may be better than the current biomarkers (i.e. transmural dispersion and instability) in identifying the risk for drug-induced potential risks for non-TdP-like VT/VF.

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References


Food and Drug Administration, HHS (2005). International Conference on Harmonisation; Guidance on 578 nonclinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals; availability. Federal Register, 70, 61133–61134.


