Original Article

Characteristics of electromechanical window in anesthetized rabbit models of short QT and long QT syndromes

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(Received April 10, 2017; Accepted July 6, 2017)

ABSTRACT — The current regulatory guidelines recommend the use of QT interval to assess the risk of arrhythmogenic potential of new chemical entities. Recently, the electromechanical window (EMW), the difference in duration between electrical and mechanical systole, has been proposed as markers for drug-induced torsades de pointes (TdP); however, data of EMW in short QT model are not available. This study aimed to characterize the EMW as a marker for drug-induced ventricular arrhythmias in anesthetized rabbit model of long QT syndrome type 2 (LQT2) and short QT syndrome (SQTS) infused with reference compounds known to lengthen or shorten QT intervals. After rabbits were anesthetized with isoflurane, body surface electrocardiograms and left ventricular pressure were recorded. The LQT2 was produced by intravenous infusion with dofetilide (n = 6), quinidine (n = 6) and sotalol (n = 6) whereas the SQTS was induced by intravenous escalating concentrations of nicorandil (n = 7), pinacidil (n = 5) and cromakalim (n = 5). The EMW in anesthetized rabbits ranged from 1.3 to 53.3 msec. All three drugs known to lengthen QT intervals prolonged QT and QTcF interval while the EMW was markedly decreased to negative values. Pinacidil significantly produced QT and QTcF shortening and significantly abbreviated the EMW (p < 0.05). This study demonstrated that the EMW is associated with QT intervals (p < 0.001). It is negative in the presence of QT-prolonging drugs while it is more positive in the presence of QT-shortening drugs. The results suggest that the EMW in anesthetized rabbits can be used in drug safety evaluation in addition to the QT interval.

Key words: Electromechanical window, Long QT, Rabbit, Safety Pharmacology, Short QT

INTRODUCTION

Torsade de pointes (TdP) and ventricular fibrillation (VF) remain a major concern for drug discovery and safety evaluation. Since those arrhythmias are very rare, the arrhythmia might slip through the safety assessment test and result in very low incidence of death in post-marketing surveillance (Hondeghe, 2006). Due to experimental complexity of using TdP and VF as the end point, the duration of QT and QTc interval has been used as the surrogate end point for ventricular arrhythmias by the pharmaceutical companies and requires by regulatory agencies (Pugsley *et al.*, 2008). However, the relationship between drug-induced arrhythmia either TdP or VF and the dura-

tion of QT/QTc interval is very poor.

It has been suggested that the predictive value of several surrogate markers is better than the predictive value of single end point assessment (Pugsley *et al.*, 2008). The EMW characterizes by the time difference between the end of electrical systole and the completion of ventricular relaxation (van der Linde *et al.*, 2010). In healthy subjects the duration of cardiac electrical and mechanical activity are closely matched so that the electrical systole (equivalent to QT interval) ends earlier than the completion of contractile relaxation, creating a positive EMW. The EMW may be measured non-invasively by the time lag between the end of QT interval and the second heart sound (Boudoulas *et al.*, 1982). A negative EMW has

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been linked to increased mortality risk in various cardiac diseases such as the coronary artery disease and mitral valve prolapse (Boudoulas *et al.*, 1981, 1982; Chambers and Ward, 1987; De Caprio *et al.*, 1984).

Recently, an electromechanical window (EMW) has been proposed as an emerging biomarker for TdP liability in anesthetized dog and guinea pig model of acquired long QT syndrome type 1 (LQT1) (Guns *et al.*, 2012a, 2012b; van der Linde *et al.*, 2010). While EMW has been shown to predict arrhythmias in the model of LQT1, it has not been evaluated in the setting of long QT syndrome type 2 (LQT2) and short QT syndrome (SQTS). Therefore, the main purpose of this study was to evaluate the characteristics of EMW as a surrogate marker for drug-induced ventricular arrhythmias in anesthetized rabbit model of LQT2 and SQTS infused with reference compounds known to lengthen or shorten QT intervals. The hypotheses of this study were (1) the EMW is negative in rabbit model of LQT2 and (2) the EMW is increased from normal value in rabbit model of SQTS.

MATERIALS AND METHODS

Animals

This study was approved by the Institutional Animal Care and Use Committee of Faculty of Veterinary Science, Chulalongkorn University (protocol number 13310072). All animal procedures were conducted in accordance with the guidelines published in *the Guide for the Care and Use of Laboratory Animals*.

Surgical procedure

A total of 41 animals, distributed equally by sex, were used. All rabbits were weighed between 2 and 2.6 kg. All rabbits were anesthetized with tiletamine/zolazepam (Zoletil, Virbac, France) 25 mg/kg, intramuscularly. After tracheotomy and intubation, the depth of anesthesia was maintained by 1.5-2.5% isoflurane with 100% oxygen. Subsequently, transthoracic electrocardiogram was recorded. A high-fidelity micromanometer catheter (Millar Instruments, Houston, TX, USA) was retrogradely advanced into the left ventricle (LV) via right internal carotid artery to determine LV pressure (LVP) signal. Intravenous catheter was positioned in the right jugular vein for drug administration. All animals were allowed to stabilize for at least 20 min after finishing the instrumentation.

Experimental protocol

Following instrumentation and hemodynamic stabilization, the steady-state electrocardiographic (RR, PQ, QRS,

and QT intervals) and LVP parameters were obtained as baseline values. In LQT2 model, groups of rabbits were each given of the following compounds: 0.1 mL/kg/min vehicle containing 0.1 M HCl in normal saline (n = 6), 10 µg/kg/min dofetilide (n = 6), 3 mg/kg/min quinidine (n = 6), 2 mg/kg/min sotalol (n = 6). In SQT model, the drugs tested in order to shorten QT interval were nicorandil (0.3, 0.5 and 1.0 mg/kg/min, n = 7), pinacidil (0.1, 0.3 and 1.0 mg/kg/min, n = 5) and cromakalim (0.001, 0.003 and 0.01 mg/kg/min, n = 5). All doses were selected from our previous publications because they are known to lengthen and shorten QT intervals (Kijawornrat *et al.*, 2006a, 2006b, 2010; Panyasing *et al.*, 2010). All doses were infused intravenously over a period of 10 min. The infusion was stopped as soon as TdP or VF started or end of the dose. All rabbits were euthanized at the end of experiment.

Drugs

Dofetilide (Pfizer, Groton, CT, USA) was dissolved in 0.9% NaCl with the help of 0.1 M hydrochloric acid to form a stock concentration of 0.1 mg/mL. Quinidine hydrochloride, nicorandil and cromakalim (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in 1% DMSO. Sotalol (Sigma-Aldrich) was dissolved in 0.9% NaCl. Pinacidil (Sigma-Aldrich) was dissolved in 5% ethanol. Analyses of dosing solutions were not performed; however, each drug was administered to rabbits within 60 min of preparation.

Data analysis and statistics

Data were obtained by using EMKA-IOX acquisition systems with sampling rates of 1000 Hz and analyzed by using ECG-auto software version 2.5.1.31 (EMKA Technologies, Falls Church, VA, USA). All ECG and LVP parameters were measured 9 min after each drug dose had been infused or 1 min before the occurrence of TdP or VF. TdP was defined as a polymorphic ventricular tachycardia where clear twisting of the QRS complexes around the isoelectric line was observed. The VF was defined as a very rapid, chaotic electrical impulses to the ventricle. The ECG intervals were measured in beats that originated from the sinoatrial node. Measurements were made from all cardiac cycle in 1 min and the average was used. The QT interval was the duration from the beginning of the Q wave to the end of T wave. The QT interval was corrected for heart rate by dividing the QT interval by the cube root of the preceding RR interval (Fridericia, 1920). The EMW was calculated as the following equation: $EMW = QLV_{Pend-QT}$. The duration of mechanical systole was expressed as the contraction time (ctrT). The

ctrT refers to the time during which the ventricles contract and eject blood into the aorta and pulmonary artery. Data are presented as mean $\pm$ standard error of mean (SEM) or medians (interquartile range, i.e. 25th – 75th percentile). In LQT2 model, comparisons between vehicle and test compounds were made using two-way ANOVA with repeated measure whereas in SQT model, the differences among escalating doses of drugs from baseline were evaluated by one-way ANOVA with repeated measure, and if indicated by a significant F-statistic, means were compared by Dunnett post-hoc analyses. Pearson and linear regression analysis were used to determine the relationship between EMW and QT interval. In all cases, $p < 0.05$ was considered as statistically significant. Receiver operating characteristic (ROC) curve analysis was performed to determine the predictive power of QT, QTcF and EMW in rabbits receiving vehicle and QT-prolonging drugs. Area under the ROC curve (AUC) and confidence intervals were calculated using commercially available software. Parameters that yielded an AUC that were greater than 0.8 were considered to have a validated predictive value for the occurrence of TdP. The optimal cut-off values were determined using Youden indexes.

RESULTS

In general, high quality electrocardiograms and left ventricular pressure were obtained from all of the rabbits. All rabbits ($n = 41$) at baseline while they were anesthetized, the recorded heart rates ranged from 178 to 288 bpm, the PQ intervals were 63.3 ± 1.4 msec, the QRS complexes were 50.6 ± 1.2 msec, the QT intervals were 163 ± 3 msec, the QTcF intervals were 257 ± 4 msec and the EMW were 21.8 ± 1.8 msec.

Effects of known QT-lengthening compounds on ECG and LVP parameters

Example tracings showing the simultaneous electrocardiogram (ECG) and left ventricular pressure (LVP) recorded at baseline and 9 min after 10 $\mu\text{g/kg/min}$ dofetilide administration are shown in Fig. 1. Effects of agents known to lengthen QTc interval are shown as percent changes from baseline in Table 1. When compared to baseline and vehicle, quinidine and sotalol but not dofetilide lengthened RR interval (i.e. decreased HR) significantly ($p < 0.05$). All QT-prolonging drugs significantly prolonged PQ interval ($p < 0.05$). Sotalol did not alter QRS complex while dofetilide and quinidine lengthened QRS complex significantly ($p < 0.05$). Plots of EMW (Fig. 2A), QT (Fig. 2B) and QTcF (Fig. 2C) versus time-points (before and after drug administration) are shown

Fig. 1. Example tracings showing the simultaneous electrocardiogram (ECG) and left ventricular pressure (LVP) recorded at baseline and 9 min after 10 $\mu\text{g/kg/min}$ dofetilide administration. The duration between the dotted line (QLVPend) and the loosely dashed line (QT interval) is the electromechanical window (EMW). The duration between the densely dashed line is the contraction time (ctrT).

Fig. 2. Plots of mean and standard error of mean for electromechanical window (EMW, A), QT interval (B) and QTcF interval (C) versus timepoints (before and after drug administration). Rabbits were exposed to vehicle (0.1 M HCl in 0.9% NaCl) or test articles known to lengthen QTcF interval (dofetilide, quinidine and sotalol). Each data point is the average of cardiac cycles for 1 min. Doses of the reference compounds were: dofetilide, 10 μ g/kg/min; quinidine, 3 mg/kg/min; and sotalol, 2 mg/kg/min. An asterisk (*) indicates $p < 0.05$ when a difference changed with statistical significance from baseline whereas # indicates $p < 0.05$ when a difference changed with statistical significance from vehicle group.

Fig. 3. Plots of mean and standard error of mean for electromechanical window (EMW, A), QT interval (B) and QTcF interval (C) versus doses of QT-shortening drugs. Rabbits were exposed to escalating concentrations of test articles known to shorten QT interval (nicorandil, pinacidil and cromakalim). Each data point is the average of cardiac cycles for 1 min. Doses of the reference compounds were: nicorandil, 0.3, 0.5 and 1.0 mg/kg/min, $n = 7$; pinacidil, 0.1, 0.3 and 1.0 mg/kg/min, $n = 5$; and cromakalim, 0.001, 0.003 and 0.01 mg/kg/min, $n = 5$. An asterisk (*) indicates $p < 0.05$ when a difference changed with statistical significance from baseline.

for groups of 6 rabbits each exposed to vehicle or test articles known to lengthen QTc interval. All test articles except vehicle significantly lengthened QT and QTcF intervals ($p < 0.05$) whereas the EMW were significantly shortened to negative values when compared to those values obtained at baseline or compared to those values obtained from rabbits receiving vehicle. Left ventricular pressure was also obtained in this study. As expected, quinidine and sotalol but not dofetilide decreased ESP, $dP/dt_{\max}$ and $dP/dt_{\min}$ significantly ($p < 0.05$) when compared to baseline and vehicle (Table 1). However, EDP was not altered by any of QT-prolonging drugs. No significant change was detected for the ctrT among groups of rabbits receiving QT-prolonging drugs and vehicle.

Effects of known QT-shortening compounds on ECG and LVP parameters

Effects of agents known to shorten QTc interval are shown as percent changes from baseline in Table 2. The durations of RR, PQ and QRS were not affected by all three doses of nicorandil, pinacidil and cromakalim. Plots of EMW (Fig. 3A), QT (Fig. 3B) and QTcF (Fig. 3C) versus doses of QT-shortening drugs are shown. Only pinacidil markedly lengthened EMW and shortened QT and QTcF intervals when compared to those values obtained at baseline ($p < 0.05$). All measured parameters of LVP (Table 2) were trivially changed in response to nicorandil and cromakalim. All three doses of pinacidil significantly reduced ESP, $dP/dt_{\max}$ and $dP/dt_{\min}$ ($p < 0.05$) when com-

Table 1. Effects of agents known to lengthen QTc interval on electrocardiograms (ECG) and left ventricular pressure (LVP) parameters.

	Baseline				Treatment			
	Vehicle	Dofetilide	Quinidine	Sotalol	Vehicle	Dofetilide	Quinidine	Sotalol
ECG Parameters								
RR (msec)	254 ± 21.2	274 ± 15.8	251 ± 9.5	251 ± 11.2	253 ± 19.7	350 ± 39.1	467 ± 70.2* [#]	412 ± 23.1* [#]
PQ (msec)	54.6 ± 2	63 ± 2.8	62 ± 2.8	64.5 ± 4.3	54 ± 2.5	71.2 ± 6.7* [#]	98.5 ± 24.5* [#]	77.2 ± 2.5* [#]
QRS (msec)	55.2 ± 2.3	53.7 ± 4.2	46.1 ± 2.7	51.6 ± 2.4	53.9 ± 2.6	63.3 ± 3* [#]	113 ± 15.1* [#]	54.2 ± 2.1
QT (msec)	166 ± 11.9	179 ± 12.8	161 ± 5.1	163 ± 7.1	166 ± 11.9	267 ± 15.9* [#]	288 ± 31.4* [#]	68.1 ± 7.6* [#]
QTcF (msec)	262 ± 12.9	275 ± 14.6	255 ± 6.2	258 ± 8.5	262 ± 13	380 ± 14.6* [#]	373 ± 24.8* [#]	367 ± 21.9* [#]
EMW (msec)	16.8 ± 3.7	23.3 ± 5.4	24.4 ± 5.2	21.6 ± 3.5	16.6 ± 3.7	-48.1 ± 11.5* [#]	-43.7 ± 15.1* [#]	-45.2 ± 18.2* [#]
LVP Parameters								
EDP (mmHg)	1.9 ± 1	2.8 ± 0.3	5 ± 2.4	-0.4 ± 1.5	1.8 ± 0.9	4 ± 0.9	11.1 ± 3.4	1.4 ± 1.7
ESP (mmHg)	56.9 ± 5.8	68.7 ± 4.9	69.4 ± 6.2	58.4 ± 5.2	56.8 ± 5.2	73 ± 4.6	44.8 ± 4.7* [#]	45.5 ± 3.4* [#]
dP/dt _{max} (mmHg/sec)	2895 ± 454	2965 ± 397	2852 ± 201	2924 ± 308	2902 ± 347	3181 ± 315	1107 ± 298* [#]	1691 ± 154* [#]
dP/dt _{min} (mmHg/sec)	-2251 ± 358	-2424 ± 186	-2665 ± 219	-2376 ± 277	-2279 ± 309	-2424 ± 133	-1209 ± 202* [#]	-1492 ± 159* [#]
ctrT (msec)	43.2 ± 0.8	44.9 ± 1	43.2 ± 0.7	43.9 ± 0.8	42.7 ± 0.8	43.3 ± 0.6	48.5 ± 3.6	45.8 ± 1.1

Data are presented as mean ± S.E.M. *compared effects of drug administration to its baseline value whereas #compared effects of drug administration among groups of known QT prolonging compounds to vehicle group, statistical difference ($P < 0.05$). QTcF, the corrected QT interval by Fridericia's formula; EMW, electromechanical window; EDP, end-diastolic pressure; ESP, end-systolic pressure; ctrT, contraction time.

Table 2. Effects of agents known to shorten QTc interval on electrocardiograms (ECG) and left ventricular pressure (LVP) parameters.

	Baseline			Treatment (Dose 1 - Low)				Treatment (Dose 2 - Medium)				Treatment (Dose 3 - High)	
	Nicorandil	Pinacidil	Cromakalim	Nicorandil	Pinacidil	Cromakalim		Nicorandil	Pinacidil	Cromakalim		Nicorandil	Cromakalim
ECG Parameters													
RR (msec)	262 ± 10.4	266 ± 19.3	222 ± 6.1	270 ± 16.1	262 ± 19.7	227 ± 8.2		267 ± 18.9	261 ± 22.5	229 ± 9.1		259 ± 17.2	256 ± 24.4
PQ (msec)	65.2 ± 3	67.1 ± 5.2	67.3 ± 5.6	63.4 ± 3.6	66.5 ± 5.2	67.4 ± 5.5		63.9 ± 3.9	64.1 ± 5.9	64 ± 1.8		64.2 ± 4.3	65.7 ± 5.5
QRS (msec)	44 ± 2.2	48.2 ± 3.1	57.4 ± 2.3	45.8 ± 2.6	48.4 ± 2.9	56.6 ± 2.7		42.6 ± 3.4	51.2 ± 3.1	57.4 ± 4.5		40.8 ± 2.9	46.6 ± 2.9
QT (msec)	159 ± 4.1	170 ± 10.5	143 ± 2.3	156 ± 6.3	168 ± 11	143 ± 2.4		151 ± 6.1	162 ± 11.1	143 ± 3.6		148 ± 4.7	115 ± 10.1*
QTcF (msec)	248 ± 4.4	264 ± 10	236 ± 4.6	241 ± 6.4	262 ± 10.8	234 ± 4.6		235 ± 5.9	253 ± 10	234 ± 5.3		232 ± 5.2	182 ± 11.5*
EMW (msec)	21.6 ± 3.9	13 ± 3.6	32.2 ± 6.8	31.5 ± 2.7	14.6 ± 2.5	34.2 ± 6.8		29.1 ± 2.8	20.6 ± 2.3	34.7 ± 9.1		25.4 ± 2.3	81.3 ± 7.5*
LVP Parameters													
EDP (mmHg)	6.7 ± 1.2	1.5 ± 1.3	0.2 ± 0.7	6.2 ± 1.1	1 ± 1.2	-0.5 ± 0.5		5.1 ± 0.9	1.4 ± 1.1	-0.6 ± 0.5		5.9 ± 1.2	3.1 ± 1
ESP (mmHg)	65.1 ± 5.2	67.6 ± 5.3	71.9 ± 5.9	46.6 ± 3.6	50.7 ± 4.2*	68.4 ± 4.8		41.4 ± 3.1	41.7 ± 2.9*	64.5 ± 4.2		43.6 ± 2.7	39.7 ± 2.9*
dP/dt _{max} (mmHg/sec)	2577 ± 383	3606 ± 299	3269 ± 413	1972 ± 341	2779 ± 196*	3177 ± 486		1769 ± 357	2174 ± 78.5*	3135 ± 581		1938 ± 288	1444 ± 68.4*
dP/dt _{min} (mmHg/sec)	-2411 ± 302	-2725 ± 218	-2929 ± 331	-1466 ± 133	-1921 ± 242*	-2700 ± 266		-1274 ± 91.2	-1514 ± 158*	-2507 ± 233		-1458 ± 92.3	-1196 ± 126*
ctrT (msec)	44.1 ± 1	42.1 ± 0.6	44 ± 0.8	42.6 ± 1.1	41.6 ± 0.4	43.6 ± 1.1		42.6 ± 1.3	41.9 ± 0.6	43 ± 1.4		41.3 ± 0.9	44.1 ± 0.5*

Data are presented as mean ± S.E.M. *compared effects of drug administration to its baseline value, statistical difference ($P < 0.05$). QTcF, the corrected QT interval by Fridericia's formula; EMW, electromechanical window; EDP, end-diastolic pressure; ESP, end-systolic pressure; ctrT, contraction time.

pared with baseline. The *ctrT* was significantly increased in response to the highest dose of pinacil. No significant change was detected for the *ctrT* among groups of rabbits receiving nicorandil and cromakalim.

Effects of test compounds on arrhythmia induction

The incidence of drug-induced TdP or VF was also recorded. The TdP occurred 2 out of 6 rabbits (33.3%) receiving intravenous infusion of dofetilide but not quinidine or sotalol. The VF occurred 1 out of 5 rabbits (20%) receiving pinacidil administration but not nicorandil or cromakalim.

Relationship between EMW and QT interval

A plot of EMW versus QT interval for 41 anesthetized rabbits that received either vehicle or drugs known to lengthen QT (i.e. dofetilide, quinidine and sotalol) or shorten QT intervals (i.e. nicorandil, pinacidil and cromakalim) is shown in Fig. 4. The relationship between EMW and QT interval was $EMW = 118 - (0.6 * QT)$, having r^2 of 0.80. This demonstrates that the relationship between the two is highly significant ($p < 0.001$).

Distribution of EMW in anesthetized rabbits

A box-and-whisker plot showing the distribution of EMW values for anesthetized rabbits receiving vehicle,

QT-prolonging drugs and QT-shortening drugs is provided in Fig. 5. The 25th percentiles for vehicle, QT-prolonging drugs and QT-shortening drugs were 15.9, -75.8 and 26.3 msec while the 75th percentiles were 32.1, -19.1 and 65.7 msec, respectively. The medians for those groups indicated by horizontal lines within the boxes were 23.6, -31.2 and 35.3 msec, respectively. There were no outliers in the data. There was statistically significant difference in the mean values of EMW among the groups (baseline vs QT-prolonging drugs, $p < 0.001$; baseline vs QT-shortening drugs, $p < 0.05$).

Predictive values of QT, QTcF and EMW in anesthetized rabbit model

ROC analysis showed that both QT and QTcF intervals had AUC values less than 0.8 while the ROC analysis of the EMW yield AUC values greater than 0.8 (Table 3). Therefore, the QT and QTcF intervals were not valid predictive values for the occurrence of drug-induced TdP. The sensitivity of the EMW was 0.8 and the specificity of the EMW was 0.7. Therefore, only EMW predicted drug-induced TdP with relatively high sensitivity and specificity. The optimal cut-off values of EMW was -32 msec (Fig. 6).

Fig. 4. Plot of averages of electromechanical window (EMW) versus QT interval for 41 anesthetized rabbits that received either vehicle (0.1 M HCl in 0.9% NaCl) or drugs known to lengthen QT intervals (i.e. dofetilide, quinidine and sotalol) or known to shorten QT intervals (i.e. nicorandil, pinacidil and cromakalim). Notice that the relationship between EMW and QT interval was very high.

Fig. 5. A box-and-whisker plot showing the distribution of electromechanical window (EMW) values for baseline, QT-prolonging drugs and QT-shortening drugs. The boxes represent the 25th to 75th percentiles, and horizontal lines within the boxes indicate the medians. An asterisk (*) indicates $p < 0.05$ and (**) indicates $p < 0.001$ compared with baseline data.

Electromechanical window in anesthetized rabbits with LQT and SQT

Fig. 6. Receiver operating characteristic (ROC) curve analysis of electromechanical window (EMW) for the prediction of dofetilide-induced torsades de pointes in anesthetized rabbits. The area under curve (AUC) is 0.82 while the optimal cut-off value of EMW was -32 msec.

Table 3. Area under the curve (AUC), sensitivity and specificity for QT, QTcF and EMW for prediction of dofetilide-induced TdP.

Parameters	AUC	Sensitivity (%)	Specificity (%)
QT	0.69	70	45
QTcF	0.63	70	45
EMW	0.82	80	70

AUC; area under the curve; QT; the QT interval; QTcF; the corrected QT interval by Fridericia's formula; EMW; electromechanical window

DISCUSSION

The main purpose of this study was to determine the characteristics of EMW in the anesthetized rabbit model of long QT and short QT syndromes. The results demonstrated that EMW shortened to negative values in rabbit model of long QT syndrome in response to all three reference compounds known to lengthen human QT intervals, and TdP was developed in dofetilide infusion. Interestingly, the EMW lengthened from baseline value in rabbit model of short QT syndrome in response to one of three reference agents known to shorten human QT intervals, and VF was developed in response to pinacidil administration.

In the present study, the EMW in anesthetized rabbits ranged from 1.3 to 53.3 msec. In response to QT-pro-

longing drugs, the QT and QTcF intervals were increased while the EMW was decreased. This is in agreement with the finding of van der Linde and colleagues (2010) and Guns and colleagues (2012b), who reported that the negative EMW is required for drug-induced TdP in a long QT syndrome type 1. Furthermore, van de Linde suggested that the appearance of a negative EMW is a prerequisite to achieve a condition in which an R-on-T induces TdP. The author had observed 200 TdP in their experiment by using a fentanyl/etomidate-anesthetized beagle dogs (van der Linde *et al.*, 2010). In the present study, the incidence of TdP was 33.3% with dofetilide infusion. Although the EMW was reduced to a negative value and statistically significant from baseline values, the EMW values of rabbits that had TdP during dofetilide infusion were not statistically different from those values of rabbits that did not develop TdP while receiving dofetilide. This may partly due to the percent of rabbits developed TdP was small so that the power was not high enough to detect the differences between groups of rabbits. Interestingly, when the ROC analysis was performed to evaluate sensitivity and specificity of QT, QTcF and EMW between rabbits that develop TdP and rabbits that did not develop TdP while receiving dofetilide, only EMW had AUC value more than 0.8 with a sensitivity of 80% and specificity of 70%. This indicated that EMW is superior to the QT and QTcF intervals for prediction of TdP in anesthetized rabbit model. However, since only dofetilide administration was able to induce TdP in this study the result of ROC analysis may be the limitation of this study. Further investigation must be performed to confirm this finding with other compounds that can produce TdP. On the other hand, previous study in halothane-anesthetized dogs (Izumi-Nakaseko *et al.*, 2014) reported that the change of EMW was only due to the alteration of the QT interval since the duration of mechanical systole was not significantly changed in response to E-4031 administration. Therefore they concluded that the sensitivity of EMW to predict the onset of TdP in response to I_{K_r} inhibitor was similar to the QT interval. The discrepancy between different conclusions among those studies may be due to the development of TdP since there is no TdP developed in the study of Izumi-Nakaseko and colleagues. This is consistent with previous report by Guns and colleagues (2012a), who found that drugs associated with prolongation of the QT interval and with well-documented TdP risk in humans (i.e. quinidine, terfenadine, dofetilide, haloperidol, domperidone and thioridazine) showed a dose-dependent prolongation of QT interval and shortening of EMW but did not evoke episodes of TdP when they were given to anesthetized guinea pigs. It has been known that blocking of both

components (i.e. slow and rapid components) of delayed rectifier potassium channels produces markedly impaired repolarization reserve (Roden, 2008; Varró and Baczkó, 2011). In LQT1 model, blockades of the slowed component of delayed rectifier potassium channel (I_{Ks}) (i.e. HMR1556, JNJ303) were used to trigger TdP (van der Linde *et al.*, 2010; Guns *et al.*, 2012b). Therefore, the difference of TdP episodes between the two models (LQT1 and LQT2) may be explained by severity of reduced repolarization reserve. The current study together with previous study (Izumi-Nakaseko *et al.*, 2014) were similar in regards to the correlation between QT and EMW. In addition, both studies demonstrated that QT-prolonging drugs did not alter the duration of ventricular systole.

The current study demonstrated that the EMW in anesthetized rabbits receiving escalating doses of pinacidil was increased from baseline while the QT and QTcF intervals were shortened. This is in agreement with our previous study in conscious dogs in which the QT interval was shortened when dogs receiving all three reference compounds (Kijawornrat *et al.*, 2010). Although doses of nicorandil and cromakalim used in the current study were higher than previous study in dogs they failed to shorten QT interval in the anesthetized rabbits. This may be due to the differences in pharmacokinetics and pharmacodynamics of drugs in different animal species (D'Alonzo *et al.*, 1994a, 1994b; Horinaka *et al.*, 2004). The finding of the current study showed that pinacidil induced VF in about 20% of the anesthetized rabbits. This is consistent with previous studies in which the use of ATP sensitive "openers" of potassium channel (I_{K-ATP}) has been shown to be associated with VF (Chi *et al.*, 1990; Lu *et al.*, 2008; Padriani *et al.*, 1992). It is known that I_{K-ATP} openers shorten action potential duration so that the effective refractory period is abbreviated (Milberg *et al.*, 2007). Pinacidil has also been shown to increase maximal transmural dispersion of repolarization between left and right ventricles in canine wedge preparation (Extramiana and Antzelevitch, 2004). These factors are known as substrates of arrhythmia mechanism (Antzelevitch and Burashnikov, 2011). In the present study, the sensitivity and specificity of QT, QTcF and EMW for prediction of VF were unable to analyze since the prevalence of pinacidil-induced VF was too low.

The use of conscious rabbit model and rabbits with heart diseases to test for torsadogenic compounds has been demonstrated previously (Kijawornrat *et al.*, 2006a, 2006b, 2012; Hamlin and Kijawornrat, 2008). The present study also demonstrated the potential utility of the anesthetized rabbit for detecting the liability of test articles to lengthen or shorten QT/QTc intervals in humans.

Three reference compounds known to lengthen human QT intervals also prolonged QT intervals in the anesthetized rabbits; and one of three reference compounds known to shorten human QT intervals also abbreviated QT intervals in this model. There are several advantages of using rabbits to test for drug-induced prolongation and/or shortening of QT intervals. These advantages may include 1) the rabbit heart shares with humans all of the transmembrane ion channels specific for controlling ventricular repolarization (Romero *et al.*, 2010) and 2) the calcium handling of human myocytes and rabbit ventricular myocytes was similar (Bers, 2002).

In conclusion, taken together, the results strongly indicated that the EMW is associated with the QT interval and it is negative in response to QT-prolonging drugs while it became more positive in response to QT-shortening drugs. In addition, the sensitivity and specificity of EMW for prediction of drug-induced TdP were in the acceptable values. Therefore, the EMW in anesthetized rabbits may be used as a marker for drug-induced TdP in response to administration of QT prolongation drugs.

ACKNOWLEDGMENTS

This study was supported by Chulalongkorn graduate scholarship to commemorate the 72nd Anniversary of His Majesty King Bhumol Adulyadej, the 90th Anniversary of Chulalongkorn University Fund (Ratchadapiseksonphot Endowment Fund – grant number GCUGR1125572060D, Special Task Force for Activating Research (GSTAR 59-002-31-002). Dr.Vudhiporn Limprasutr was partly supported by the Graduate School, Chulalongkorn University (Overseas Research Experience Scholarship for Graduate Student) to perform research while she was at QTest Labs and the Support for the Overseas Presentations of Graduate Level Academic Thesis to present poster at Safety Pharmacology Society Annual Meeting.

Conflict of interest---- The authors declare that there is no conflict of interest.

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