การใช้ค่าอิเล็กโทรเมคานิคอลวินโดว์เป็นตัวชี้วัดความเสี่ยงในการเกิดภาวะหัวใจห้องล่างเสียจังหวะ
โดยใช้กระต่ายเป็นต้นแบบ

นางสาววุฒิพร ลิ้มประสูตร

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THE USE OF ELECTRO-MECHANICAL WINDOW AS A SURROGATE MARKER FOR PREDICTING THE RISK OF VENTRICULAR ARRHYTHMIAS IN RABBIT MODELS

Miss Vudhiporn Limprasutr

A Dissertation Submitted in Partial Fulfillment of the Requirements
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วุฒิพร ลิ้มประสูตร : การใช้ค่าอิเล็กโทรเมคานิคอลวินโดว์เป็นตัวชี้วัดความเสี่ยงในการเกิดภาวะหัวใจห้องล่างเสียจังหวะโดยใช้กระต่ายเป็นต้นแบบ (THE USE OF ELECTRO-MECHANICAL WINDOW AS A SURROGATE MARKER FOR PREDICTING THE RISK OF VENTRICULAR ARRHYTHMIAS IN RABBIT MODELS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. น.สพ. ดร. อนุศักดิ์ กิจถาวรรัตน์, 144 หน้า.

การศึกษานี้มีสมมติฐานว่า 1) การลดลงของค่าอิเล็กโทรเมคานิคอลวินโดว์จนเป็นลบในกระต่ายตัวแบบของกลุ่มอาการคิวทียาวชนิดที่ 2 มีความสัมพันธ์กับการเกิดทอซาด เดอ ปวง และการเพิ่มของค่าอิเล็กโทรเมคานิคอลวินโดว์ในกระต่ายตัวแบบของกลุ่มอาการคิวทีสั้น เกี่ยวข้องกับการเกิดหัวใจห้องล่างสั่นระริก 2) อิเล็กโทรเมคานิคอลวินโดว์เป็นตัวบ่งชี้ที่มีความน่าเชื่อถือในการทำนายการเกิดหัวใจห้องล่างสั่นระริกที่เกิดจากกล้ามเนื้อหัวใจขาดเลือดในกระต่ายตัวแบบ 3) ค่าอิเล็กโทรเมคานิคอลวินโดว์อาจมีค่าน้อยกว่าในกระต่ายที่ได้รับแคลเซียมแชนแนลบล็อคเกอร์ก่อนที่จะให้ยาโดเฟทิไลซึ่งทำให้เกิดทอซาด เดอ ปวง และ 4) อิเล็กโทรเมคานิคอลวินโดว์เป็นค่าที่อาจไม่ได้รับผลกระทบจากการเปลี่ยนพรีโหลด อัตราการเต้นของหัวใจ ความดันโลหิต และความแรงของการบีบตัวของกล้ามเนื้อ เพื่อทดสอบสมมติฐานเหล่านี้ การศึกษาแบ่งออกเป็น 4 ส่วน การศึกษาส่วนที่ 1 เพื่อประเมินลักษณะของอิเล็กโทรเมคานิคอลวินโดว์ในกระต่ายตัวแบบของกลุ่มอาการคิวทียาว และคิวทีสั้น ผลการศึกษาแสดงให้เห็นว่าอิเล็กโทรเมคานิคอลวินโดว์สามารถใช้เป็นตัวบ่งชี้เพื่อประเมินความปลอดภัยของยาได้ เนื่องจากมีค่าเป็นค่าลบระหว่างการได้รับยาที่ทราบว่ายืดระยะคิวทีออก ในขณะที่ค่ามีการเพิ่มขึ้นเมื่อได้รับยาที่หดระยะคิวทีให้สั้นลง การศึกษาส่วนที่ 2 มีวัตถุประสงค์เพื่อพิจารณาการใช้ค่าอิเล็กโทรเมคานิคอลวินโดว์ในการทำนายหัวใจห้องล่างสั่นระริกที่เกิดจากกล้ามเนื้อหัวใจขาดเลือดในกระต่ายที่ผูกหลอดเลือดแดงโคโรนารี ผลการวิจัยชี้ให้เห็นว่าการเพิ่มขึ้นของค่าอิเล็กโทรเมคานิคอลวินโดว์มากกว่า 64 มิลลิวินาที และการเพิ่มขึ้นของค่าความแปรปรวนระยะสั้นของคิวทีมากกว่า 5.31 มิลลิวินาที สามารถใช้เป็นตัวบ่งชี้ในการทำนายหัวใจห้องล่างสั่นระริกในกระต่ายที่ถูกเหนี่ยวนำให้เกิดกล้ามเนื้อหัวใจขาดเลือด การศึกษาที่ 3 มีวัตถุประสงค์เพื่อประเมินลักษณะของอิเล็กโทรเมคานิคอลวินโดว์ในกระต่ายต้นแบบของการเหนี่ยวนำให้เกิดทอซาด เดอ ปวงด้วยโดเฟทิไล ผลการทดลองพบว่าโดเฟทิไลลดค่าอิเล็กโทรเมคานิคอลวินโดว์ลงเป็นค่าลบทั้งในกระต่ายที่มีทอซาด เดอ ปวง และไม่มีทอซาด เดอ ปวง ซึ่งในกลุ่มที่มีทอซาด เดอ ปวง ค่าอิเล็กโทรเมคานิคอลวินโดว์มีค่าเป็นลบมากกว่า วัตถุประสงค์ของการศึกษาภาคที่ 4 คือเพื่อหาผลกระทบจากพรีโหลด อัตราการเต้นของหัวใจ ความดันโลหิต และความแรงของการบีบตัว ผลการทดลองพบว่าในกระต่าย ค่าอิเล็กโทรเมคานิคอลวินโดว์ได้รับผลกระทบจากการเปลี่ยนแปลงอัตราการเต้นของหัวใจ และความแรงในการบีบตัว แต่ไม่ได้รับผลกระทบจากพรีโหลดและความดันโลหิต ดังนั้นการตีความการเปลี่ยนแปลงอิเล็กโทรเมคานิคอลวินโดว์จึงควรระมัดระวัง สรุปได้ว่าค่าอิเล็กโทรเมคานิคอลวินโดว์สามารถใช้เป็นตัวบ่งชี้สำหรับทำนายทอซาด เดอ ปวงค และหัวใจห้องล่างสั่นระริกในกระต่ายตัวแบบของกลุ่มอาการคิวทียาว คิวทีสั้น และหัวใจห้องล่างสั่นระริกในกระต่ายตัวแบบกล้ามเนื้อหัวใจขาดเลือด อย่างไรก็ตามอิเล็กโทรเมคานิคอลวินโดว์ได้รับผลกระทบจากการเปลี่ยนแปลงอัตราการเต้นของหัวใจและความแรงในการบีบตัว

ภาควิชา สรีรวิทยา ลายมือชื่อนิสิต .....

สาขาวิชา สรีรวิทยาการสัตว์ ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2559

5475409231 : MAJOR ANIMAL PHYSIOLOGY
KEYWORDS: ELECTROMECHANICAL WINDOW / VENTRICULAR ARRHYTHMIAS / RABBITS / TORSADES DE POINTES / VENTRICULAR FIBRILLATION / QT INTERVAL

VUDHIPORN LIMPRASUTR: THE USE OF ELECTRO-MECHANICAL WINDOW AS A SURROGATE MARKER FOR PREDICTING THE RISK OF VENTRICULAR ARRHYTHMIAS IN RABBIT MODELS. ADVISOR: ASST. PROF. DR. ANUSAK KIJTAWORNRAT, D.V.M., Ph.D., 144 pp.

This study hypothesized that 1) a reduction of electromechanical window (EMW) to negative value in rabbit's model of long QT syndrome type 2 is associated with torsade de pointes (TdP) and an increase of EMW from baseline value in rabbit's model of short QT syndrome is associated with ventricular fibrillation (VF); 2) the EMW is a reliable marker for predicting ischemia-induced VF in rabbit model; 3) the value of EMW may be less negative in rabbits receiving calcium channel blocker before administration of dofetilide to induced TdP; and 4) EMW is a marker that may not be affected by changing of preload, heart rate (HR), blood pressure (BP) and contractility. In order to test these hypotheses, the study was divided into 4 parts. The study part 1 aimed to evaluate the characteristics of EMW in animal models of LQT and SQT syndromes. The results showed that EMW can be used as a biomarker for drug safety evaluation as it was negative during infusion of known QT prolonging agents while it became more positive during infusion of known QT shortening drugs. The study part 2 aimed to determine the use of EMW for predicting ischemia-induced VF in anesthetized rabbits produced by coronaries ligation. The results suggested that the increasing of EMW >64 ms as well as the elevation of STV_{QT} >5.31 ms can potentially be used as biomarkers for predicting of VF in anesthetized rabbits with myocardial ischemia. The study part 3 aimed to assess the characteristic of EMW in the rabbit model of dofetilide-induced TdP. The results showed that dofetilide decreased EMW to negative values both in rabbits with (TdP+) and without (TdP-) TdP development in which it decreased more for TdP+ group. The purpose of study part 4 was to determine the effect of preload, HR, BP and contractility on EMW. The results indicated that, in the anesthetized rabbit model, EMW was affected by changing of HR and contractility but not preload and BP. Therefore, interpretation of changes of EMW in this model should be cautious. In conclusion, EMW can be used as a surrogate marker for predicting the TdP and VF in LQT, SQT anesthetized rabbit models and in ischemia-induced VF rabbit model. However, the EMW was affected by extremely changes of HR and contractility.

Department: Veterinary Physiology Student's Signature .....
Field of Study: Animal Physiology Advisor's Signature .....
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ABBREVIATION LIST

ANOVA	Analysis of variance
APD	Action potential duration
AS	Atherosclerosis
AUC	Area under the ROC curve
bpm	Beat per minute
BrS	Brugada syndrome
CETP	Cholesteryl ester transfer protein
ctrT	Contraction time
dP/dt_{max}	Maximum rate of rise of LVP
dP/dt_{min}	Maximum rate of fall of LVP
EADs	Early afterdepolarizations
ECG	Electrocardiogram
EDP	End-diastolic pressure
EMW	Electro-mechanical window
Epi	Epicardium
ESP	End-systolic pressure
HDLs	High-density lipoproteins
hERG	Human ether-a-go-go-related gene
HR	Heart rate
hr	Hour(s)
I_{CaL}	L-type calcium current
iCEB	Index of cardiac electrophysiological balance
ICH	International Conference on Harmonization
I_{K1}	Inward rectifier potassium current
$I_{K\text{-}ATP}$	ATP-sensitive potassium current
I_{Kr}	Rapid component of delayed rectifier potassium ion current
I_{Ks}	Slow component of delayed rectifier potassium ion current
I_{Kur}	Ultra-rapid delayed rectifier like potassium current
I_{to}	Transient outward potassium current

kg	Kilogram(s)
LAD	Left anterior descending artery
LDLs	Low-density lipoproteins
LQT	Long QT syndrome
LQT1	Long QT syndrome type 1
LQT2	Long QT syndrome type 2
LQT3	Long QT syndrome type 3
LVP	Left ventricular pressure
M	Midmyocardium
MAP	Monophasic action potential
mg	Milligram(s)
MI	Myocardial infarction
min	Minute(s)
ml	Milliliter(s)
ms	Millisecond(s)
NCEs	New chemical entities
NCX	Sodium calcium exchanger
PE	Phenylephrine
QLVPend	The beginning of Q wave to the end of LVP
QTc	Corrected QT
QTcB	Corrected QT by Bazett's formula
QTcF	Corrected QT by Fridericia's formula
relT	Relaxation time
ROC	Receiver operating characteristic
RyR	Ryanodine receptor
s	Second(s)
SBP	Systolic blood pressure
SEM	Standard error of mean
SNP	Sodium nitroprusside
SQT	Short QT syndrome
SR	Sarcoplasmic reticulum

STV_{QT}	Short-term variability of QT interval
Tau	Relaxation time-constant
TDR	Transmural dispersion of repolarization
TdP	Torsades de pointes
TQT	Thorough QT
TRIaD	Triangulation, reverse use dependence, instability and dispersion
VF	Ventricular fibrillation
VPC	Ventricular premature complex
WHHL	Watanabe heritable hyperlipidemic

CHAPTER I
INTRODUCTION

Previous reports have shown that several drugs have been withdrawn from the market because of proarrhythmic liability such as sudden cardiac death, ventricular fibrillation (VF), and torsades de pointes (TdP) (Lasser et al., 2002). Interestingly, the list of withdrawal drugs has included both cardiovascular (e.g. disopyramide, dofetilide, sotalol) and noncardiovascular drugs (e.g. astemizole, cisapride, terfenadine). Nearly all cases, drugs caused arrhythmias by blockade of the rapid component of delayed rectifier potassium ion channel (I_{Kr}) encoded by the human ether-a-go-go-related gene (hERG) (Sanguinetti et al., 1995). Blocking of I_{Kr} results in lengthening of ventricular repolarization duration and produces a prolongation of the QT interval on electrocardiogram (ECG).

Drug-induced long QT syndrome (LQT) is characterized by QT interval prolongation and increased risk of TdP. Torsades de Pointes is described as a polymorphic ventricular tachycardia where QRS complexes twist around an isoelectric line (Dessertenne, 1966) (Fig. 1). Symptoms of TdP include palpitations, syncope, and seizure-like activity. TdP is usually self-terminated but may degenerate into VF and sudden cardiac death. Since a variety of medications have been implicated in drug-induced LQT, the lengthening of QT interval and TdP are the most common reasons for pharmaceuticals to restrict or remove drugs from the market (Lasser et al., 2002).

Figure 1. Torsades de Pointes (TdP) is characterized by the occurrence of rapid polymorphic ventricular tachyarrhythmias with a twisting morphology of the QRS complex around the isoelectric line.

A major concern of drug-induced QT prolongation is a potential of drugs to delay ventricular repolarization and develop polymorphic ventricular arrhythmias. Regulatory agencies have developed guidelines for preclinical and clinical evaluations of new chemical entities (NCEs). Those guidelines are aimed to investigate ventricular repolarization and thorough QT interval prolongation. The QT interval is the electrocardiographic manifestation of ventricular depolarization and repolarization. It is measured from the beginning of the QRS complex to the T wave termination (Fig 2). The lengthening of QT interval has been used as a surrogate biomarker for the risks of drug-induced ventricular arrhythmias especially TdP. On the other hand, the shortening of QT interval has been reported to associate with VF (Brugada et al., 2004).

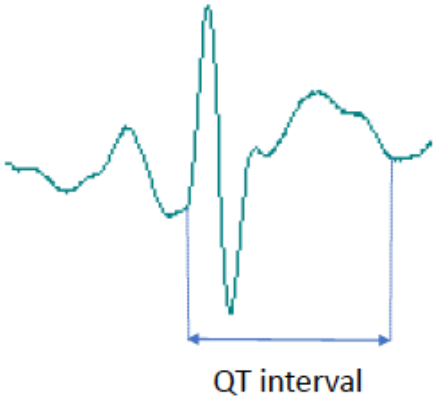


Figure 2. Example of normal ECG trace with QT interval labeled from anesthetized rabbits.

QT intervals may vary due to diurnal effects, sex hormones, hERG blocking drugs, body temperature, genetic defects, electrolyte imbalance, autonomic fluctuations, heart rate, ECG acquisition technique, as well as intra- and interobserver variability (Morganroth et al., 1991; Molnar et al., 1996; Jonsson et al., 2010; Ruan et al., 2010; Krishnan et al., 2012). The change of heart rate plays an important role on the duration of QT interval. QT intervals normally shorten when heart rate accelerates and lengthen when heart rate decreases. Therefore, a rate corrected QT (QTc) interval

should be used to normalize the heart rate. The most frequently used QTc formulas are Bazett and Fridericia equations (Bazett, 1920; Fridericia, 1920) (Table 1).

Table 1

Corrected QT (QTc) calculated by Bazett's and Fridericia's formula

Method	Formula
Bazett	$QTcB = QT/\sqrt{RR}$
Fridericia	$QTcF = QT/(RR(0.33))$

Although prolongation of QT and QTc intervals have been used as a marker for the risk of arrhythmia development utilized by both pharmaceutical companies and regulatory agencies, the poor relationship between QT/QTc intervals and the incidences of arrhythmias particularly TdP has been reported progressively. For example, terfenadine blocks I_{Kr} and prolongs QT/QTc interval minimally but has been reported to produce unacceptable high incidence of TdP (Quirk et al., 2002). Haloperidol causes QTc interval lengthens only 5-7 ms, but TdP is occurred in humans when receiving a therapeutic dose of haloperidol. Interestingly, several drugs (e.g., salbutamol, ebastine, tamoxifen, carvedilol) are associated with QT prolongation but not considered proarrhythmic; therefore, the use of only QT interval as biomarker for drug-induced arrhythmias is highly questionable.

Recently, the prolongation of QT/QTc intervals has been suggested that it is not a marker for torsadogenic risk but it actually is an effect of antiarrhythmia. In fact, the increase of transmural dispersion of repolarization (TDR), the change in triangulation, reverse use dependence, instability and dispersion (TRIaD) are a better marker of TdP (Hondeghem et al., 2001; Antzelevitch et al., 2004; Thomsen et al., 2004). Although the TDR and TRIaD are associated with arrhythmias in patients with congenital and acquired LQT, additional preclinical and clinical studies are needed to assess the use of those indices for predicting the arrhythmic risk of NCEs.

More recently, van der Linde has demonstrated that electro-mechanical window (EMW) is associated with the incidence of TdP in anesthetized dog model of

long QT syndrome type 1 (LQT1) and is superior than the use of QT/QTc interval (van der Linde et al., 2010b). In healthy subjects, the duration of cardiac electrical and mechanical activity are closely matched. The EMW has been used to investigate the harmonization of the electrical and mechanical work. It represents the time different between the duration of left ventricular pressure (LVP) measured from the beginning of Q wave to the end of LVP (QLVPend) and the QT interval. Normally, the electrical systole (equivalent to QT interval) ends earlier than the completion of contractile relaxation, creating a positive EMW (Fig 3). The EMW can 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, formerly referred to as inversed QT/QS2 ratio or 'QT>QS2 Syndrome', occurs if either LV contraction duration shortens or if the QT interval prolongs. It has been linked to increased mortality risk in various cardiac diseases, such as prolonged QT in the coronary artery disease and mitral valve prolapse, and can be pronounced during an increased autonomic tone (Boudoulas et al., 1981a; Boudoulas et al., 1982; De Caprio et al., 1984; Chambers and Ward, 1987). Recent investigation in anesthetized dogs demonstrated that an invasive EMW markedly decreased to -109±6 ms is related to development of TdP, which was prevented by atenolol and verapamil (van der Linde et al., 2010b). In anesthetized guinea pigs, EMW was decreased to a negative value by known torsadogenic drugs (e.g. haloperidol, domperidone, terfenadine, dofetilide, thioridazine and quinidine). In the same model, salbutamol and diltiazem, known non-torsadogenic compounds, did not change the EMW (Guns et al., 2012b). More recently, the study in the LQT patients showed that mean EMW was significantly more negative in the LQT-mutation carriers than in controls (-60±59 ms versus 4±27 ms). This value was even more negative in symptomatic than in asymptomatic mutation carriers (-75±68 ms versus -39±36 ms) (ter Bekke and Volders, 2012). In summary, these findings strongly imply that the EMW appears to possess a reliable biomarker for arrhythmic risk in addition to QT/QTc intervals; however, more experimental work is necessary to examine the use of EMW in other animal models.

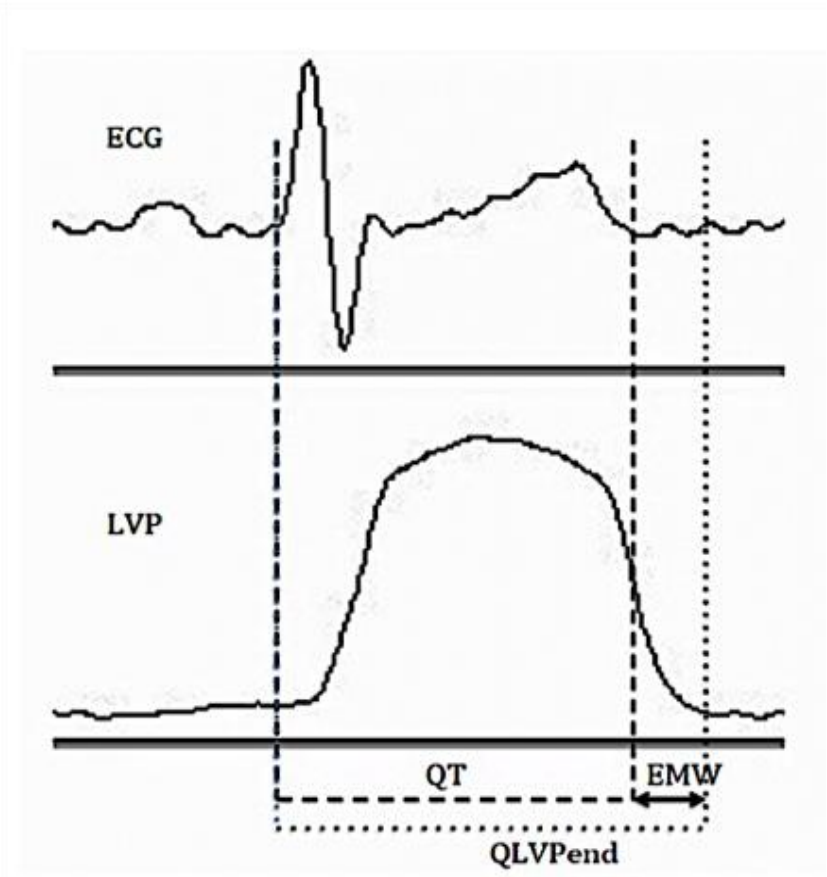


Figure 3. The electro-mechanical window (EMW) is shown. The relationship between the electrocardiogram (ECG) and left ventricular pressure (LVP) are demonstrated. The time difference between the QT interval and QLVPend is the EMW.

Rabbits are another animal model that is widely used for cardiovascular diseases (Duranthon et al., 2012; Peng, 2012). The rabbit is a particularly relevant small animal model since the regional patterns of myocardial deformation, cardiac cell electrophysiology, heart size to excitation wavelength ratio, coronary architecture and response to ischemia or pharmacological interventions are much closer to human than small rodents (Harken et al., 1981; Panfilov, 2006; Nattel et al., 2008; Burton et al., 2012). The rabbit is also an important model for investigations of arrhythmogenesis and pharmacological safety testing (Hondeghem, 2016).

Therefore, the objectives of this study were as follow:

1. To evaluate the usefulness of EMW as a surrogate marker for assessment the risk of arrhythmias by compared with QT, QTc, and short-term variability of QT interval (STV_{QT}) in rabbit models of LQT type 2 and short QT syndrome (SQT)
2. To evaluate the usefulness of EMW as a surrogate marker for assessment the risk of arrhythmias by compared with QT, QTc, and short-term variability of QT interval (STV_{QT}) in rabbit models of ischemia-induced VF
3. To evaluate the characteristics of EMW by compared with QT, QTc, and STV_{QT} on the prevention of drug-induced TdP
4. To evaluate effects of preload, contractility, blood pressure, and heart rate on the EMW

The hypotheses of this study were as follow:

1. A reduction of EMW to negative value in rabbit models of LQT type 2 is associated with TdP and an increase of EMW from normal value in rabbit models of SQTS is associated with VF.
2. The EMW is a reliable marker for predicting ischemia-induced ventricular fibrillation in rabbit models.
3. The EMW may be a less negative value in the dofetilide-induced TdP rabbit receiving calcium channel blocker.
4. The EMW is a marker that may not be affected by changing of preload, contractility, blood pressure, and heart rate.

This study would provide more information concerning the valuable of the EMW as an alternative biomarker for detecting proarrhythmic risk (i.e. VF, TdP) of drugs in anesthetized rabbits.

CHAPTER II
LITERATURE REVIEW

The incidences of drug-induced cardiac arrhythmias, especially TdP and VF, are commonly interfered with drug development (Hondeghem et al., 2007). During 1975 to 1999, a total of 548 NCEs were approved to use in humans. Surprisingly, 10.2% of those approved drugs were withdrawn from the market in the US and other countries around the world. It has been reported that the most common reasons for withdrawal problem are related to QT interval prolongation and torsade de pointes arrhythmias (Lasser et al., 2002). For example, terfenadine was showed to induce QT prolongation and TdP (Pratt et al., 1996). Sparfloxacin has been removed from the US because of the tremendously prolonged ventricular action potential (Hagiwara et al., 2001). Thioridazine possessed the highest risk for QT interval prolongation among the patients receiving psychiatric medications (Reilly et al., 2000). Cisapride was stopped marketing in 2000 due to 117 cases of QT interval prolongation and 107 cases of TdP (Wysowski et al., 2001). The estimate risk of TdP from the use of quinidine is approximately 1.5% (Roden et al., 1986). Bepridil, a calcium channel blocker, was withdrawn from the US market in 2003 due to prolongation of QT interval and develop TdP (Izumi et al., 2010).

These early findings have led to regulatory recommendations to detect a QT prolongation early during drug development. The principles of Safety Pharmacology were established to characterize the pharmacodynamic/pharmacokinetic relationship of a drug's adverse effects using continuously evolving methodology (Bass et al., 2004). While toxicological studies examine the high-dose adverse events of a compound, Safety Pharmacology aims at determining potential side effects on vital and secondary functions when the drug is given at acute therapeutic or supratherapeutic doses. Instructions for carry out properly preclinical safety studies were given by the International Conference on Harmonization (ICH) in a set of guidelines: ICH S7A which deals with the three vital functions on the central nervous, the cardiovascular, and the respiratory systems, and ICH S7B which focuses more specifically on the proarrhythmic risk assessment related to the QT interval prolongation. These preclinical guidelines were followed more recently by the ICH E14 recommendations for conducting thorough QT (TQT) investigations in early clinical trials.

According to the ICH S7B and ICH E14, a recent survey of pharmaceutical industry practice, articles of opinion, and guidelines from regulatory authorities describe a core battery of three preclinical assays typically used to predict torsadogenic potential in man (Gralinski, 2000; Hammond et al., 2001). These include (1) an *in vitro* assay investigating the inhibitory potential of a compound on I_{Kr}; (2) an *in vitro* repolarization assay evaluating changes in action potential duration (APD) in an integrated electrophysiological system (such as Purkinje fiber or papillary muscle); and (3) an *in vivo* assay in a non-rodent species evaluating changes in the QT interval of the ECG. The recommendations include characterization of drug effects on individual ion currents (e.g. I_{Kr}) as well as conduct a TQT study in volunteers, with therapeutic dose, a supratherapeutic dose or a therapeutic dose with a metabolic inhibitor. The goal of the TQT is to detect the degree of QT prolongation attributable to peak serum concentrations of either the active drug or its metabolites. A focus on toxicity screening early in the drug development process may save time, and prevent or minimize adverse clinical outcomes. Therefore, the QT prolongation is considered a surrogate for TdP-liability. Recently, substantial evidence suggests that change of QT interval by itself generates not only false positives, but also false negative in terms of drug-induced arrhythmias (Shah and Hondeghem, 2005).

A. Torsades de pointes (TdP)

TdP is a polymorphic ventricular tachycardia (i.e. the morphology of the QRS complexes is variable and not constant) (Dessertenne, 1966). It was associated with prolongation of the QTc interval on the ECG. Symptoms of TdP are primarily related to the rapid heart rate resulted in reduced blood pressure and cardiac output which include palpitations, dizziness, lightheadedness, shortness of breath and syncope (Tisdale et al., 2011). In some cases, TdP may be non-sustained and terminated spontaneously. However, TdP often degenerates rapidly into VF, resulting in sudden cardiac death. There are reports of more than 100 drugs including antibiotics, antidepressants, and cardiovascular drugs that may cause QTc interval prolongation and TdP. Risk factors for TdP include congenital and acquired QT prolongation,

advanced age, female sex, hypokalemia, hypomagnesemia, hypocalcemia, and bradycardia (Roden, 2008). Torsades de Pointes was frequently associated with APD prolongation; however, TdP was also occurred without change of APD and even with APD shortening (Hondeghem, 2016). More importantly, not all QT-prolonging drugs were associated with proarrhythmia, to the contrary QT-prolongation can even be antiarrhythmia (Shah and Hondeghem, 2005).

Although QT prolongation is an essential first step in TdP, it is usually not considered sufficient to induce TdP. QT interval prolongation is particularly proarrhythmic when associated with increased dispersion in the recovery of excitability (Han and Moe, 1964). Additional steps appear to be required for TdP to occur, for example, the development of early afterdepolarizations (EADs) that are calcium mediated and were identified as a cause of reentrant arrhythmias in *in vitro* models (Jackman et al., 1988). The ability to block or disrupt the hERG potassium channel, thereby reducing I_{Kr} and prolonging the QT interval, is a common feature of drugs that induce TdP.

Intravenous administration of magnesium sulfate is the initial therapy of choice. Drugs known to prolong the QTc interval should be discontinued immediately. Serum potassium and/or magnesium should be replaced if the patient is hypokalemic or hypomagnesemic. Overdrive pacing is highly effective in preventing recurrence of TdP or in case of refractory to magnesium sulfate. Isoproterenol may be used in case of no pacing available; however, it is contraindicated in ischemic heart diseases or patients with congenital LQT (Gupta et al., 2007)

B. Ventricular fibrillation (VF)

VF is characterized by a heartbeat with rapid and erratic electrical impulses and has been reported to be associated with Brugada syndrome (BrS) (Antzelevitch et al., 2005; Mizusawa and Wilde, 2012; Antzelevitch and Patocskai, 2016). The BrS was a hereditary condition involving idiopathic ventricular tachycardia, VF and sudden cardiac death in structurally normal hearts. Traditionally, BrS has been linked to loss of function mutations in the SCN5A gene, which encodes for the cardiac Na^+ channel.

Other than BrS, SCN5A mutations have been associated with sick sinus syndrome, progressive cardiac conduction defect and idiopathic VF without BrS findings (Akai et al., 2000; Benson et al., 2003). By contrast, gain of function SCN5A mutations are observed in LQT type 3 (Wang et al., 1995). BrS and LQT share many similarities, existing in congenital or acquired forms (Havakuk and Viskin, 2016).

Furthermore, during the acute phase of myocardial infarction (MI), studies have suggested that 3-12% of all MI cases develop VF resulting in sudden cardiac arrest. The pathophysiology of VF during MI is complex and is associated with both environmental and genetic causes (Spooner et al., 2001; Dekker et al., 2006; Kaikkonen et al., 2006; Bezzina et al., 2010; Deo and Albert, 2012; Marsman et al., 2014; Jabbari et al., 2015). The VF in MI is most likely resulted from the interaction of multiple factors, including ischemia, hemodynamic alterations, electrolyte imbalance, reentry mechanism, genetic, and environmental triggers (Volpi et al., 1987; Brezins et al., 1996; Mehta et al., 1997; Gheeraert et al., 2000). Acute obstruction of the coronary flow affects the resting membrane potential and alters the inward and outward ionic currents, lead to alterations in conduction, refractory period, and automaticity (Janse and Wit, 1989). Experimental studies have found that there are different phases of MI in which VF occurs (Janse and Wit, 1989; Clements-Jewery et al., 2005). The first phase occurs between 2-10 min after occlusion and the second phase occurs from 12-30 min after occlusion.

C. Long QT syndrome (LQT)

LQT is one of the most important causes of syncope and sudden death due to ventricular arrhythmias such as TdP development (Priori, 2014). The QT interval prolongation may be congenital or acquired. At present, there are 15 subtypes of congenital LQT which associated with mutations on a different gene (Alders and Christiaans, 1993). Most diseases causing mutations are located in genes encoding for repolarizing K^+ channels or depolarizing Na^+ channels and may be modified proteins that related to the transfer of channels to the membranes (Tester and Ackerman, 2014). The most common form of congenital LQT is caused by mutations in KCNQ1,

which encodes the pore-potassium channel subunit term Kv7.1, underlying the slow component of the delayed rectifier potassium channel (I_{Ks}). The I_{Ks} is a slowly activating and prominent during the plateau and repolarizing phases of the cardiac action potential. Missense mutations within the transmembrane pore region are associated with a dominant-negative effect on ion channel function (>50% reduction in function) and a high-risk phenotype (Moss et al., 2007). Expression of I_{Ks} has been demonstrated in both human atrial and ventricular myocytes (Wang et al., 1994; Li et al., 1996; Jost et al., 2005). In addition, it has also found in non-human species including dogs and rabbits (Liu and Antzelevitch, 1995; Varro et al., 2000; Stengl et al., 2006). On the other hand, I_{Ks} is expressed at very low levels or absent in mouse hearts (Xu et al., 1999). Insufficient I_{Ks} activation in LQT1 results in failure to counterbalance the calcium influx, prolonging the action potential and increasing susceptibility to arrhythmia.

The second type of congenital LQT is mutations in KCNH2 (also termed hERG) encoding the Kv11.1 potassium channel. The KCNH2 mutation leads to defective hERG protein, resulting in a decrease in the I_{Kr}. Similar to LQT1, missense mutations in the transmembrane pore region (S_5-pore-S_6) or in the N-terminus have been determined to give higher arrhythmogenic risk than mutations in non-pore (S_1-S_4) or C-terminal regions in long QT type 2 (LQT2) (Moss et al., 2007; Nagaoka et al., 2008). Irrespective of the underlying cause, a decrease in I_{Kr} delays repolarization of the cardiac action potential and prolongs the QT interval on the ECG. Together, mutations in genes whose expression leads to I_{Kr} and I_{Ks} account for 80-90% of congenital LQT.

Type 3 congenital LQT (LQT3) is caused by mutations in the cardiac sodium channel, encoded by SCN5A (Wang et al., 1995). While patients with K^+ channel-associated LQT are generally at higher risk or cardiac events during exercise, patients with LQT3 have elevated risk at rest (Schwartz et al., 2001). The LQT3 is a disease of impaired $Na_v1.5$ inactivation. In most cases, impaired inactivation is manifest as a sustained non-inactivating inward current, or late current (I_{NaL}).

In some cases, the mutations decrease I_{Kr} or I_{Ks} or alter SCN5A fast inactivation, while in other cases; the functional defects involve other potassium channels (the inward rectifier channel or the acetylcholine gated potassium channel) or increase inward current through L-type calcium channels (I_{CaL}) (Schwartz and Volders, 2014).

The examples of other subtypes of congenital LQT are LQT type 4 or Ankyrin-B syndrome, LQT type 7 or Andersen-Tawil syndrome type 1, LQT type 8 or Timothy syndrome. Ankyrin-B is an adaptor protein that functions in the cytoskeletal network to ensure proper localization and stabilization of ion channels and transporters. Timothy syndrome is caused by gain-of-function mutations in the CACNA1C gene, which encodes $Ca_v1.2$, the pore forming subunit of a cardiac voltage-gated calcium channel.

The acquired LQT caused by exposure to drugs that prolong the duration of the ventricular action potential or secondary to cardiomyopathies such as dilated or hypertrophic cardiomyopathies (Sipido et al., 2000; Bednar et al., 2001). The Kv11.1 is a major drug target in drug-induced LQT. Most drugs that block I_{Kr} channel bind to the open state of the hERG channel at the inner pore thereby preventing permeation of potassium (Mitcheson et al., 2000; Zheng et al., 2010). Some drugs, including pentamidine and probucol, produce a reduction in I_{Kr} by reducing trafficking of hERG protein to the plasma membrane, leading to prolongation of the QT interval (Cordes et al., 2005; Kuryshev et al., 2005; Guo et al., 2007b). Ketoconazole blocks hERG directly and decrease hERG channel density at the cell surface (Takemasa et al., 2008). This observation led to regulatory recommendations to screening NCEs for 'hERG block'. In some cases, drug-induced LQTSreduced cell surface expression of the channel (Kuryshev et al., 2005). Several other pharmacological agents induced LQT in susceptible patients via modulation of other important ionic currents (e.g., I_{Na}, I_{Ks}) (Veerman et al., 2013; Yang et al., 2014).

It has been shown that female sex is an independent risk factor for development of drug-induced TdP in patients in which approximately 65-75% of TdP cases were occurred in women (Lehmann et al., 1996; Drici et al., 1998; Locati et al., 1998). There is no statistical difference in the QTc interval between men and women before puberty (Merri et al., 1989; Stramba-Badiale et al., 1995). The gender differences in QTc intervals appear to be correlated with age-dependent changes in serum levels of sex hormones. Recent study showed that the repolarization in men was most influenced by the effect of testosterone on calcium currents. Female sex hormones are also involved with the gender differences both in QTc intervals and in the

susceptibility of TdP. During the menstrual cycle and pregnancy in females, there are dynamic fluctuations in QT interval and TdP risk which may correlate with changes in serum levels of ovarian steroids (Nakagawa et al., 2006). The gender differences in drug-induced LQT are partly based on the electrical differences resulting from expression of various ion channels. In humans, decrease expression for several repolarizing channels including hERG was observed in both male and female (Gaborit et al., 2010). In addition, estrogen has also reported to augment membrane trafficking of hERG by enhancing interaction to heat shock proteins in non-genomic pathway (Anneken et al., 2016). In guinea pig ventricular myocytes, it has been revealed that physiological concentrations of estrogen acutely delayed cardiac repolarization, resulting in a slight prolongation of the APD and QTc interval (Kurokawa et al., 2008). Recently, study in an aromatase knockout mouse as an *in vivo* non-estrogen model supports the effects of estrogen on cardiac electrophysiology (Kurokawa et al., 2015).

In addition to the prolongation of cardiac APD due to the disease causing alterations of cardiac ion channel function, alterations in Ca^{2+} handling properties of the cardiomyocytes are causatively linked to EADs formation in LQT. The reactivation of $I_{Ca,L}$ currents is thought to be a major causative factor for the initiation of EADs that can trigger TdP (January and Riddle, 1989). Additionally, other Ca^{2+} handling proteins such as the ryanodine receptor (RyR), the sodium calcium exchanger (NCX), the sarcoplasmic reticulum ATPase 2a pump, phospholamban, and the Ca^{2+}-calmodulin-dependent protein kinase-II may contribute to or prevent the formation of EADs by altering cytoplasmic and sarcoplasmic reticulum (SR) Ca^{2+} concentrations, spontaneous Ca^{2+} release, and Ca^{2+} transient. An increased intracellular Ca^{2+} concentration or spontaneous SR Ca^{2+} release via the RyR may thereby activate NCX in its forward mode, which in turn prolongs APD and causes the re-activation of the window I_{CaL} current or the re-activation of I_{Na} in late phase 3 repolarization thus creating the EADs (Bers, 2008; Priori and Chen, 2011). In transgenic LQT2 rabbits, abnormalities in Ca^{2+} handling properties such as an increased spontaneous SR Ca^{2+} release via a phosphorylated, hyperactive RyR have been identified that may further promote EADs/arrhythmogenesis (Terentyev et al., 2014).

In addition to the role of Ca^{2+} on LQT-related arrhythmogenesis, changes in Ca^{2+} handling properties may be causatively linked to the observed mechanical dysfunction in LQT. An increased Ca^{2+} influx via I_{CaL} and accompanying with increased Ca^{2+}-induced Ca^{2+}-release from the SR during the prolonged APD may increase the cytoplasmic Ca^{2+} concentration and prolong Ca^{2+}-transient duration (Bassani, 2006). However, detailed mechanisms underlying the observed mechanical dysfunction in LQT still warrant further investigation.

D. Short QT syndrome (SQT)

In academic, pharmaceutical companies and regulatory agencies, they are looking signals of lead compounds that prolong QT interval while little attention has been paid to QT shortening signal. Nevertheless, several experts expressed their concerns that drug-induced QT shortening might be equally important (Lu et al., 2008b; Shah et al., 2010). The SQT is a rare, inheritable channelophathy of the heart characterized by abnormally short QT intervals, which increases ventricular arrhythmias in the absence of structural heart disease (Gussak et al., 2000; Gaita et al., 2003). The ECG is the main diagnostic method of SQT. More recent clinical description of congenital forms of SQT has raised concerns on the potential risk associated with drug-induced QT shortening. The SQT may precipitate ventricular tachycardia or VF. In 1993, clinical data suggested that patients with a shortened average QTc interval possess a higher risk for sudden cardiac death than patients with normal QTc interval (Algra et al., 1993).

The mechanisms and physiological processes causing QT interval changes are numerous. Theoretically, APD shortening may result from facilitation of repolarization currents such as I_{Kr}, I_{Ks}, inward rectifier potassium current (I_{K1}) or ATP-sensitive potassium current ($I_{K\text{-}ATP}$), or from reduced I_{Na} or L-type calcium current (I_{CaL}) currents. Most of data on QT shortening and related arrhythmias have been obtained from studies using K-ATP channels openers. The *ex vivo* investigations using rabbit Purkinje fibers, Langendorff-perfused rabbit hearts, or canine ventricular wedge preparations have clearly demonstrated how several K-ATP channel openers (pinacidil, levcromakalim or nicorandil) produced APD abbreviation, QT interval shortening and

increased transmural dispersion of repolarization (TDR) (Extramiana and Antzelevitch, 2004; Milberg et al., 2007b; Lu et al., 2008b).

The relationship between short QT interval and arrhythmia initiation is much less understood than that of long QT interval and TdP. It has been studied in related specific genetic abnormalities, such as the BrS. In these cases, the non-uniformity of the ECG patterns supports the thought that repolarization heterogeneity plays a major role in SQT and BrS (Antzelevitch and Fish, 2001).

The congenital SQT may be classified according to the affected genes controlling the myocardial ion channels, although a few data from genetic screening has been reported (Mazzanti et al., 2014). The classification is also according to the affected ion channels. The underlying abnormalities of the SQT may be distinguished as follow: the first, SQT due to the gain of function of potassium channels and the second, SQT due to the loss or suppression of the function of calcium channels. Because of heterogeneous contribution of repolarizing ion currents within the heart, there are the inhomogeneity and dispersion of repolarization, which provide substrate for the development of VF.

The SQT subtype 1 is caused by mutations in KCNH2 (hERG) (Brugada et al., 2004). Two missense mutations have been described that lead to gain in function and shortening of the APD. The SQT subtype 2 is caused by mutations in KCNQ1, causing a gain of function of I_{Ks}, resulting in an abbreviation of the APD and shortening of the QT interval in vitro (Bellocq et al., 2004; Brugada et al., 2004). In 2005, SQT subtype 3 was discovered that it was associated with a gain of function in the KCNJ2 gene, encoding for the rectifying channel protein Kir2.1 (Priori et al., 2005). Functional characterization of the mutation demonstrated a significant increase in the outward I_{k1} current and strongly suggested that myocardial tissues should be capable of sustaining stable functional reentry at high frequencies (Priori et al., 2005). Loss of function mutations in genes encoding the cardiac I_{CaL} (i.e. CCNA1C and CACNB2b) was reported (Antzelevitch et al., 2007). SQT subtype 6 was related to mutation in the CACNA2D1 gene (Hong et al., 2005).

The majority of compounds that shortened QT interval are potassium rectifier activators. These agents increase the I_{Kr} current and have demonstrated VF in the

isolated Langendorff perfused heart (Grunnet et al., 2008). On the other hand, these compounds have been shown to suppress EADs and decrease the repolarization heterogeneity in several tissue preparations (Hansen et al., 2006). Suppression of QT interval prolongation was also reported with vasodilators and benzothiazine derivatives that act as openers of ATP-sensitive potassium channels (Testai et al., 2010). However, while the shortened APD was established with pinacidil, it is questionable whether it leads to QT interval shortening beyond the underlying heart rate change (Wu et al., 2005; Kijtawornrat et al., 2010). Antiepileptic drugs that affecting the voltage-gated sodium channels may lead to QT interval shortening (Dixon et al., 2008; Kropeit et al., 2015). The mechanism for this effect is not entirely understood but may possibly be due to the analogous effects of mild pure sodium channel blockers (i.e. class IB antiarrhythmic drugs). Drug-induced shortening of QT intervals by selective Kv1.5 inhibitor has also been described. This compound was suggested to be a heart rate dependent (Ford et al., 2016).

E. Acute myocardial ischemia (MI)

Acute MI generates several electrophysiological effects including action potential shortening (Janse and Wit, 1989). Canine and feline ventricular epicardium has been found to be more sensitive than endocardium to action potential shortening during acute ischemia or metabolic inhibition (Kimura et al., 1987; Taggart et al., 1988). The difference was also observed in rabbit heart (Wolk et al., 1998). There is evidence that the I_{Ca} is depressed more by ischemia in the epicardium than in the endocardium (Kimura et al., 1991). Hypoxia reduces transient outward potassium (I_{to}) current and activation of K_{ATP} channels (Miyoshi et al., 1996; Verkerk et al., 1996). It has also been suggested that the presence of I_{to} in the epicardium might amplify ischemia-induced changes in other currents, even if I_{to} itself is not directly affected (Lukas and Antzelevitch, 1993).

During early ischemia (<10 min), slow ventricular conduction is mainly due to a decrease in I_{Na} resulting from impaired membrane depolarization (Kleber et al., 1987). Consequently, during ischemia, a premature impulse may cause a unidirectional block, leading to arrhythmias by re-entry mechanism. Importantly, V_{max} in ventricular

endocardium has been shown to be less sensitive to depression than in the epicardium. This marked electrical heterogeneity could be responsible for ischemia-induced ventricular arrhythmias.

The ST segment is the portion of the ECG that lies between the end of the QRS complex and the beginning of the T wave. Normally, the ST segment stays at the same level as the TP segment. There are two changes of the ST segment that considered clinically important: the ST segment displacement and the change in ST segment morphology. The ST segment displacement, either upward (elevation) or downward (depression) of more than 1 mm from baseline, is abnormal.

The ST segment elevation is the so-called 'injury current'. It has been explained as the current flow from the injured myocardium, where the cells are partially depolarized, to the uninjured myocardium. In animal experiments, however, the injury current measured by using a direct current coupled amplifier, causes TP segment depression rather than ST segment elevation (Kleber et al., 1978). The depression or loss of the action potential in the epicardium, but not in endocardium, would result in a transmural voltage gradient during repolarization that could manifest as ST segment elevation. This mechanism likely perhaps contribute importantly to that observed in acute myocardial ischemia (Yan and Kowey, 2000).

F. Current biomarkers for drug-induced arrhythmias

Electrophysiological markers associated with drug-induced TdP other than APD and QT interval prolongation include but are not limited to: triangulation; reverse use dependence; temporal, spatial and transmural dispersion of ventricular repolarization (TRIaD); the difference in duration between the peak and end of the T wave (T_{peak}-T_{end}) (an index of transmural dispersion of repolarization, TDR), STV_{QT}, EMW, and index of cardiac electrophysiological balance (iCEB).

However, all measurements are affected by both technical and biological limitations. They were used to evaluate the associations between the studied parameters and the occurrence of ventricular arrhythmias.

a. QT/QTc interval

The entire period of ventricular depolarization and repolarization is represented by the interval from the beginning of Q wave to the end of the T wave in ECG, known as the QT interval. As ventricular repolarization becomes prolonged, the ventricle becomes more susceptible to premature electrical impulses or EADs that can trigger TdP (January and Riddle, 1989). Therefore, the longer the QT interval, the greater the possibility of TdP. However, TdP can occur despite a normal or even a shortened QT interval (Hondeghem, 2008a; Hondeghem, 2011). Moreover, QT interval has a low sensitivity and specificity for several reasons such as difficulty and inaccuracy in determining the end of the T wave, alteration by both autonomic tone and heart rate.

The QT intervals are varying when the heart rate changes. As the heart rate increases, the QT interval shortens and vice versa. Therefore, the QT interval must be corrected for heart rate variations. The heart rate-adjusted QT interval is known as the QTc interval. Several heart rate formulae exist and the most commonly used is Bazett's formula ($QTcB = QT/\sqrt{RR}$). In bazett's formula, the constant QTc is the QT interval when RR interval is 1 (i.e. the heart rate is 60 bpm). However, it is only useful for a narrow range of heart rates. As a result, it will significantly over-correct for fast heart rates and under-corrects for slow heart rates (Davey, 2002; Isbister and Page, 2013). The Fridericia's formula ($QTcF = QT/\sqrt[3]{RR}$) is expected to be better; however, it is still problematic for fast heart rates. Practically, there is no QT correction formula that fits for all subjects. There is an increasing research interest in QT correction for each individual's ECG (Malik et al., 2002). However, this approach is impractical for routine clinical ECG recording. Firstly, a large amount of data is needed for each subject. This is clearly time consuming and expensive. Secondly, this approach fails to incorporate some important physiological aspects of the QT interval, such as the effects of quickly adaptation of the QT interval to changes in heart rates (i.e QT memory).

b. TRIaD

Disturbances of triangulation (T), reverse use dependence (R), instability (I) and dispersion (D) of the cardiac action potential (TRIaD) have been shown to be highly

predictive of proarrhythmia and confirmed by numerous authors (Hondeghem et al., 2001); (Guo et al., 2007a; Milberg et al., 2007b; Dumotier et al., 2008).

Triangulation is derived from a qualitative assessment of the action potential. In the presence of certain drugs that delay repolarization, the shape of the action potential may appear triangle. Triangulation results from a reduction in outward repolarizing currents and/or an increase of depolarizing inward currents during fast repolarization. Triangulation has been defined as the duration from APD_{30} to APD_{90} (Shah and Hondeghem, 2005). There are at least four reasons why the slowing of phase 3 repolarization (triangulation) might potentially concern. First, increasing the time spent in the voltage window for the calcium current can trigger EADs (January et al., 1991). Second, increasing the time in the voltage window for the sodium current can similarly yield EADs (Katzung et al., 1975). Third, during the final part of the repolarization, the sodium channels recover from inactivation so that provides more time for the currents to trigger EADs or reentry arrhythmias. Fourth, because not all the cardiac APD are identical, it is important that many potassium channels are open at the end of phase 3 of the action potential. This not only clamps the membrane potential closer to the potassium equilibrium potential but also reduces the tissue impedance. For the above reasons, it is expected that a prolongation of the APD by triangulation will be more supportive for the occurrence of TdP. Triangulation can occur with prolongation, no change or even shortening of the APD. Several experimental observations in isolated rabbit hearts demonstrated that triangulation is highly proarrhythmic (Hondeghem et al., 2001; Martin et al., 2006; Guo et al., 2007a; Milberg et al., 2007a). Two studies highlight important aspects of triangulation. First, both terodiline and tolterodine block hERG channels in sub-therapeutics concentrations; however, one has been shown to associate with TdP while the other not. Terodiline caused triangulation without APD prolongation while tolterodine prolonged the APD without triangulation. Therefore, blockade of hERG does not necessarily be a proarrthymic (Martin et al., 2006). Second, QT prolongation was consistently observed when quinolone was administered in isolated rabbit hearts. However, only heart that exhibited triangulation and dispersion developed TdP (Milberg et al., 2007b). Consistent with previous studies, amiodarone was identified in

the SCREENIT system with a trend for triangulation, but no reverse-use dependence, no instability, no EADs and no TdP (Hondeghem and Hoffmann, 2003; Hondeghem et al., 2003).

Reverse use dependence of compounds described as drugs that delay repolarization, the prolongation of the action potential is greater at low HR compared with high HR. Therefore, it reflects the increased likelihood of TdP to occur at low HR. According to the reverse use dependence definition, most class III antiarrhythmic agents prolong the APD at normal HR, but as the HR increases the prolongation decreases (Hondeghem and Snyders, 1990).

Instability is an unstable of APD, yielding the oscillations. Due to long and short oscillations of APD, the excitatory wave leads to TdP. Recently, beat-by-beat instability of APD strongly predicted TdP, but QT prolongation did not (Thomsen et al., 2004). The importance of instability of repolarization was also illustrated for atrial fibrillation (Gong et al., 2007).

c. Short-term variability of QT interval (STV_{QT})

Recently, beat-to-beat variability or STV_{QT} was proposed as an additional parameter to quantify arrhythmic risk (Thomsen et al., 2004). It is a measure of temporal dispersion, which is a variation in repolarization between beats. A Poincare plot is generated by plotting QT of each beat versus QT of the preceding beat. The STV_{QT} is calculated as the distance of the points in the plot to the line of identity, averaged over 30 consecutive beats. The formula may express as $STV_{QT} = \sum \left| D_{n+1} - D_n \right| /[30x\sqrt{2}]$; D = the duration of QT (ms). The STV_{QT} in *in vivo* is the macroscopic counterpart of instability as measured under in the *in vitro* conditions.

Currently, laboratories in academia and the pharmaceutical industries have intensively investigated the predictive value of STV_{QT}. In anesthetized guinea pigs, the study of STV_{QT} differentiated four torsadogenic drugs at therapeutic concentrations (Fossa et al., 2004). Measurement of STV_{QT} can easily be applied to human ECGs. Recently, patients with a history of drug-induced arrhythmias had a higher STV_{QT} compared to healthy control group (Hinterseer et al., 2006). An increased QT variability

had an association with increased incidence of malignant ventricular arrhythmias in 871 post-infarction patients with severe left ventricular dysfunction (Haigney et al., 2004).

To further explore the relation between proarrhythmia and STV_{QT}, the effects of several proarrhythmic drugs on STV_{QT} were assessed. The QTc and STV_{QT} were evaluated before the drug induced ectopic beat and compared to their baseline values. In the chronic atrio-ventricular block dog model, TdP can be induced by numerous I_{Kr}-blockers. After dofetilide, the drug prolonged the QTc interval and increased STV_{QT} prior to TdP. When the drugs were separated by proarrhythmic outcome, the STV_{QT} was found to increase only in the group where TdP occurred, while QTc prolonged in both groups (Thomsen et al., 2007). Moreover, there was no significant increase in STV_{QT} and no TdP at a clinically relevant dose of sertindole. At high dose of sertindole, the STV_{QT} was increased and the TdP was occurred in 76% of the population (Thomsen et al., 2006). Thus, drug-induced TdP is associated with an increase in STV_{QT}.

Although the ionic and cellular mechanisms that underlie the generation of STV_{QT} are not fully understood, the study of this parameter may serve as an appropriate approach toward prediction of clinical outcomes.

d. Electro-mechanical window (EMW)

In the 1980s, the electro-mechanical coupling, the relationship between the duration of electrical systole (indirectly measured as the QT interval) and the mechanical systole (indirectly measure as the Q wave to the second heart sound; QS_2) has been studied extensively in humans (Boudoulas et al., 1981b). In healthy individuals, the duration of the QT interval during the normal range of heart rate is parallels to duration of the QS_2. The inversion is called $QT>QS_2$ syndrome and has been used as an index for mitral leaflet prolapse, the Romano-Ward inherited LQT and coronary artery disease (Boudoulas et al., 1982; Chambers and Ward, 1987; Vincent et al., 1991). However, the heart is not fully relaxed at the second heart sound, thus the mechanical systole is not completed

Electrical and mechanical cardiac function are connected via electro-mechanical coupling mechanisms and mechano-electrical feedback, suggesting that an impairment of electrical function should have impact on mechanical cardiac function (Pfeiffer et al., 2014). The 2D, M-mode echocardiography was the first method used to assess mechanical alterations in LQT patients (Nador et al., 1991). In addition, it provides information on regional contractility/relaxation and tissue deformation of the heart. Using these techniques, it was demonstrated that LQT patients possess a prolonged and heterogeneous duration of myocardial contraction. The rabbit models of LQT2 have a noticeably impaired diastolic relaxation also (Haugaa and Edvardsen, 2011; Odening et al., 2013).

In LQT patients, the duration of contraction was longer when compared with healthy controls (Haugaa et al., 2009; Haugaa et al., 2010). Moreover, the symptomatic LQT patients had even longer duration of contraction than asymptomatic LQT patients. Ter Bekke et al. (2015) suggested the use of EMW negativity, the duration of LV mechanical systole assessed by continuous wave Doppler echocardiography minus QT interval, as an additional parameter besides QTc interval to improve risk stratification in LQT patients. The EMW was found to be negative in LQT patients while being positive in healthy controls. It was particularly negative in symptomatic LQT patients (ter Bekke et al., 2015).

Recently, van der Linde has demonstrated that EMW is associated with the incidence of TdP in anesthetized dog model of LQT1 (van der Linde et al., 2010b). The EMW is used to investigate the harmonization of the electrical and mechanical work. It represents the time difference between the duration of left ventricular pressure measured from the beginning of Q wave to the end of left ventricular pressure (QLVPend) and the QT interval. In fentanyl/etomidate-anesthetized dogs (FEAB), a negative EMW to -109±6 ms is related to the development of TdP, which was prevented by atenolol and verapamil (van der Linde et al., 2010b). The authors also reported that change in body temperature was not affecting the EMW whereas decreased HR may slightly decreased EMW (van der Linde et al., 2010b).

In anesthetized guinea pigs, EMW was decreased to a negative value by known torsadogenic drugs (e.g., haloperidol, domperidone, terfenadine, dofetilide,

thioridazine, quinidine) whereas salbutamol and diltiazem, the known negative controls for torsadogenic drugs did not change the EMW (Guns et al., 2012b). The EMW appears to possess a reliable biomarker for arrhythmic risk; however, only 2 species (dogs and guinea pigs) have been used to evaluate this index. Furthermore, the animal models that have been used to investigate EMW are limited to LQT1 and LQT2.

e. Transmural dispersion of repolarization (TDR)

Recent studies from a number of laboratories have delineated three distinct cell types in the ventricular myocardium: epicardial, midmyocardial (M cells) and endocardial cells (Belardinelli et al., 2003; Antzelevitch, 2004b). The M cells have a lesser distribution of I_{Ks}, a larger late Na^+ current and a larger Na^+/Ca^{2+} exchange current when compared with epicaridal and endocardial cells (Antzelevitch, 2004b). These manifests as the differences in action potential configuration and duration, refractory periods and responses to pharmacological intervention (Liu et al., 1993). Enhanced TDR leads to increased heterogeneity of tissue refractoriness, which provides a substrate for re-entrant arrhythmia (Antzelevitch, 2007).

TDR results from heterogeneous expression of ion channels across the ventricular wall and influences T-wave morphology on the ECG (Antzelevitch, 2007). Cells from the mid-myocardium are repolarized later than endocardium or epicardium and tend to have less I_{Ks} (Burashnikov and Antzelevitch, 2005). As a consequence, a given reduction in I_{Kr} has a greater effect on mid-myocardial repolarization than on that in other regions of the ventricular wall. Under physiological conditions, the M cells exhibit the longest APD in the left ventricular myocardium, which makes them vulnerable to agents that prolong APD (Poelzing and Rosenbaum, 2005). The action potential duration of the epicardium determines the peak of the T wave whereas the action potential duration of the M cell, determines the end of the T wave. Therefore, the $T_{peak} - T_{end}$ interval has been proposed as a direct measure of TDR (Fig 4) (Yan and Antzelevitch, 1998).

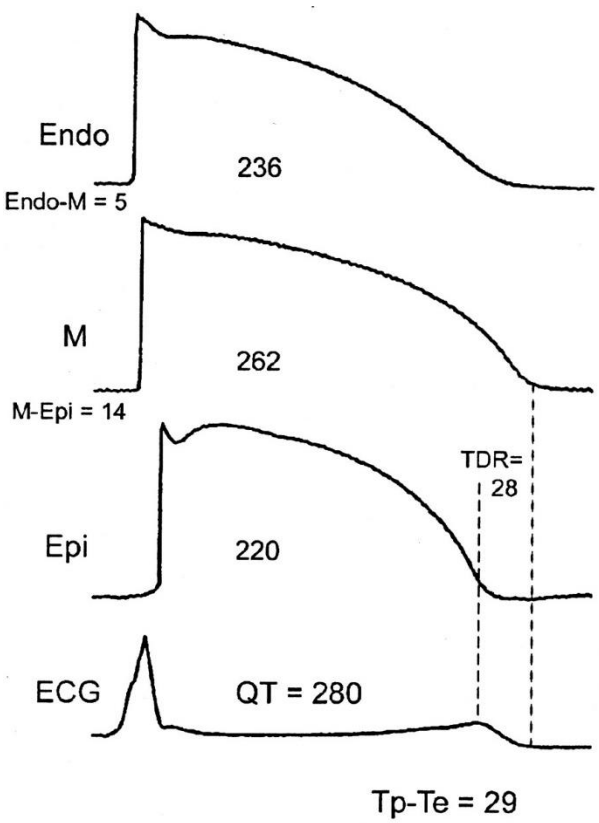


Figure 4. Transmural sequence of canine left ventricular wedge preparation. Epicardial (Epi) and M cell (M) action potentials and a transmural ECG were shown. TDR is defined as Tp-Te. Picture was modified from recently study (Fish et al., 2004).

Recent studies have provided guidelines for the estimation of TDR when the T wave morphologies of ECG are complexes, including negative, biphasic, and triphasic T waves (Emori and Antzelevitch, 2001). With these complexes, the interval form the nadir of the first component of the T wave to the end of the T wave provides an accurate electrocardiographic approximation of TDR. The $T_{peak} - T_{end}$ is also lead-dependent because the dispersion of repolarization varies with different cardiac regions.

Some agents (i.e. amiodarone and sodium pentobarbital) and syndromes prolong the QT interval but do not cause an increase in TDR (Sicouri et al., 1997; Shimizu et al., 1999). These agents and conditions generally do not induce TdP suggesting that QT prolongation is not a determinant for the development of

arrhythmias (Antzelevitch, 2005). Although additional studies are clearly needed to evaluate the utility of these non-invasive indices of electrical heterogeneity and their prognostic value in the assignment of arrhythmic risk the evidence is accumulating in support of the hypothesis that TDR rather than QT prolongation underlies the substrate that is responsible for the development of TdP (Belardinelli et al., 2003; Antzelevitch, 2004a; Antzelevitch, 2004b; Fenichel et al., 2004).

The *in vitro* studies show that hERG blocking agents (e.g. dl-sotalol) cause action potential prolongation and slow ventricular repolarization (Yan et al., 2001). Once the heart exposed to dl-sotalol, all action potentials prolong within the ventricle. However, the M cell action potentials prolong more than the action potentials of the Epi or endocardium so that the T wave is widened. The prolongations of the M cell action potentials cause a greater separation of action potential durations between all cell types (i.e. epicardium, endocardium, Purkinje fibers) resulting in an increase in TDR. In general, a drug that has a profile similar to the dl-sotalol (i.e. producing an increase in TDR) may be considered as a proarrhythmic drug. On the other hand, a drug that tends to reduce TDR may be considered as an antiarrhythmic agent. The hypotheses developed from this model are based on many marketed drugs including azimilide and amiodarone in which they did not increase TDR or induce EADs (Yan et al., 2001; Belardinelli et al., 2003; Antzelevitch, 2004b). Pentobarbital sodium is another agent that has been shown to produce a dose-dependent prolongation of the QT interval accompanied by a reduction in TDR (Shimizu et al., 1999). The TdP is not observed under these conditions, nor can it be induced with programmed electrical stimulation. Amiodarone and pentobarbital have the ability to block I_{Ks}, I_{Kr} and late I_{Na}. This combination produces a preferential prolongation of the APD of epicardium and endocardium so that the QT interval is prolonged whereas the TDR is actually reduced and TdP does not occur.

Consequently, the difference between T_{peak} and T_{end} is taken as a measure for TDR in the *in vivo*. (Antzelevitch et al., 1999; Anyukhovsky et al., 1999). A recent extensive simulation study has shown that the peak of the T wave does not coincident with the moment of epicardial repolarization (Clayton and Holden, 2004). Moreover, the $T_{peak} - T_{end}$ varies with species and heart rate. It also has a significance inter-

individual variability (Gupta et al., 2008). It was found that normalizing the $T_{peak} - T_{end}$ with the QT interval (i.e. $T_{peak} - T_{end}$/QT) has a relatively constant normal range between 0.17 and 0.23. Although this index has a controversial it has been shown to predict arrhythmic risk in LQT and distinguish patients with TdP from those without TdP (Takenaka et al., 2003). It has also been demonstrated to predict arrhythmic risk or mortality in BrS and other clinical conditions such as ST elevation in MI, diabetes mellitus, and pediatric sepsis (Gupta et al., 2008).

f. Index of Cardiac Electrophysiological Balance (iCEB)

The iCEB has been proposed and characterized by the QT interval divided by QRS duration (Fig. 4) (Lu et al., 2013). This index has demonstrated utility in predicting cardiac arrhythmias after administration of drugs such as dofetilide, digoxin, and isoprenaline in rabbit perfused-wedge preparations. It was subsequently validated in humans with drugs infusion, LQT and BrS (Robyns et al., 2016).

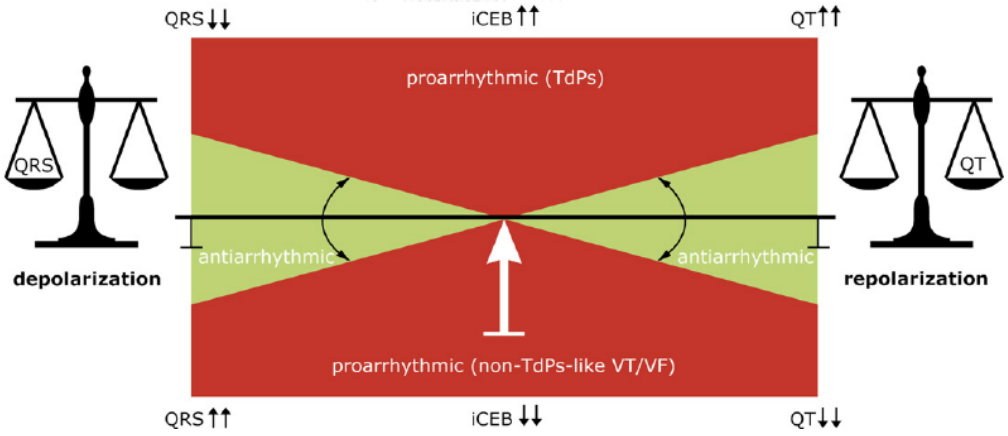


Figure 4. Balance and imbalance of the depolarization (QRS duration) and repolarization (QT interval). Large changes of index of cardiac electrophysiological balance (iCEB) or imbalance of cardiac electrophysiology (red area) may lead to arrhythmic events (Lu et al., 2013).

Recently, Tse proposed that iCEB should be modified from QT/QRS to the following formulas: ($T_{peak} - T_{end}$)/QRS and $T_{peak} - T_{end}$/(QT x QRS) (Tse, 2016; Tse and

Yan, 2016). This is based on pre-clinical findings that increased the TDR is a proarrhythmic factor, and it was superior to the QTc in arrhythmic risk stratification (Castro-Torres et al., 2015).

G. Rabbit models of ventricular arrhythmias

The high morbidity and mortality of cardiovascular diseases require appropriate animal models for translational research. It is not only to better understand the underlying mechanisms but also to explore corresponding targets and drugs for clinic treatment. Rabbits are another animal model that is widely used for cardiovascular diseases (Duranthon et al., 2012; Peng, 2012).

The rabbit is a particularly relevant small animal model since the regional patterns of myocardial deformation, cardiac cell electrophysiology, heart size to excitation wavelength ratio, coronary architecture and response to ischemia or pharmacological interventions are much closer to human than small rodents (Harken et al., 1981; Panfilov, 2006; Nattel et al., 2008; Burton et al., 2012). The rabbit is also an important model for investigations of arrhythmogenesis and pharmacological safety testing (Hondeghem, 2016).

The value of the rabbit ventricular arrhythmia and pro-arrhythmia models is largely depended on their predictive capability to humans. Human relevance is determined by the electrophysiological background of rabbit ventricular myocytes compared with humans and other frequently used animal preparations for experimental arrhythmias or drug testing (Varro et al., 1993). Mice and rats are commonly used in arrhythmia research and to create transgenic LQT models. However, mice and rats have a limited value for studies on repolarization. The rodents have a triangular action potential waveforms while humans, dogs and rabbits have a prominent plateau phase and a rectangular action potential (Saito et al., 2009). The main repolarizing currents in mice and rats are I_{to} and ultra-rapid delayed rectifier like potassium currents (I_{Kur}) whereas dogs, rabbits and humans use I_{kr}. Acute administration of I_{Kr} blockers exert no effect on ventricular action potential in mice and rats while it prolongs repolarization time in humans, dogs and rabbits. Moreover, rabbit transgenic LQT model may have more relevance to humans when compare with murine

transgenic LQT models. The function and gating of different types of potassium channels are similar in rabbits and humans, with the exception to I_{to}. The Kv1.4 channels are responsible for I_{to} in rabbits that have slower recovery from inactivation than the Kv4.3 channels expressed in human ventricle (Wang et al., 1999). In rabbits, I_{Ks} and I_{Kr} show higher current densities with similar gating kinetics compared to those in humans. The I_{Kr} is the main phase 3 repolarizing current, which is very similar in both rabbits and humans. Therefore, rabbits are more useful than rats or mice for electrophysiological and pharmacological antiarrhythmic and pro-arrhythmic investigations. A recent study found that I_{Ks} densities were lower in humans than in rabbits and dogs suggesting a weaker repolarization reserve in humans (Jost et al., 2013). The kinetics of rabbit I_{Ks} is more similar to human compared to those of guinea pigs or dogs (Jost et al., 2013).

None of the currently used preclinical methods have been proven to be fully predictive for the torsadogenic potential of NCEs in man. The *in vivo* methods introduce an intrinsic degree of variability needed for QT correction for heart rate. On the other hand, thee methods explore potential effects of active metabolites as well as neurogenic and hormonal influences. The *in vitro* methods lack the ability to provide data on pharmacodynamics.

a. The isolated rabbit hearts (SCREENIT)

The *in vitro* techniques can be employed to better understand the relationship between prolongation of APD and torsadogenic risk. Recently, an isolated rabbit heart model was developed. It was aimed at reproducing conditions that are associated with an increased TdP occurrence. In this system, monophasic action potentials were simultaneously recorded from up to 8 sites evenly spread in a circular pattern around both ventricles. TdP was produced with several QT prolonging agents and occurrence of EADs was used to predict TdP arrhythmia.

Moreover, the correlation between APD prolongation and proarrhythmia in isolated rabbit heart preparations were investigated (Hondeghem et al., 2001; Hondeghem and Hoffmann, 2003). In this model, the bundle of His is sectioned, the heart is stimulated and the monophasic action potential is recorded from the left

ventricle epicardium and subendocardium. For torsadogenic compounds, the APD prolongation was accompanied by proarrhythmia indices such as TRIaD, spatial and temporal dispersion.

The multiple monophasic action potential (MAP) recordings mean that it is not critical for all electrodes to remain in place for duration of the experiment. In addition, studies using left or right ventricular endocardial or epicedial MAP recordings and ECG from rabbit isolated hearts have demonstrated how I_{Kr} activators and $I_{K\text{-}ATP}$ openers may shorten the QT interval, increase the TDR, and enhance the ventricular vulnerability to VF (Milberg et al., 2007a; Lu et al., 2008a).

The isolated heart model described as SCREENIT, allows high-throughput and is now used in several companies for early drug screening in the clinical candidate selection phase (Hondeghem et al., 2001; Valentin et al., 2004). The SCREENIT is a fully automated computerized screening model designed to discriminate between pro- and antiarrhythmic compounds (Hondeghem et al., 2001). The hypothesis behind this model is that action potential prolongation is not the only factor that predisposes the heart to arrhythmogenesis. Hondeghem (2001) has proposed the TRIaD biomarker for prediction of arrhythmia by using this model. In this model, MAP recording electrodes are placed on epicardial and endocardial surfaces to record TRIaD elements. The $APD_{10\text{-}90}$, conduction velocity, and proarrhythmic events such as EADs are recorded. The SCREENIT model and the TRIaD concept had been tested in three validation studies. The aim was set to determine if the model could discriminate between proarrhythmia and antiarrhythmic properties of compounds. It is also aimed to determine the predictability of this model to TdP in man. These validation studies have included a range of pro-, anti- and nonarrthymic drugs and vehicles. The conclusions from these studies are: i) using SCREENIT and TRIaD analysis, proarrhythmic drugs were correctly categorized, ii) TRIaD elements precede overt proarrhythmia and iii) the TRIaD elements probably act synergistically as a single occurrence. To date, over 16,000 experiments have been conducted including approximately 300-blinded tests of 70 widely used clinical drugs with both torsadogenic and non-torsadogenic properties (Hoffmann and Warner, 2006).

In addition to their utility in the study of arrhythmogenesis and arrhythmia maintenance, the rabbit heart preparations can also be used as a tool to study the mechanisms of induction and effective termination of arrhythmias by electric shocks. The rabbit heart is a commonly used model to study the mechanisms of fibrillation and termination and has been utilized in a series of studies over the past two decades (Himel and Knisley, 2007). In a Langendorff rabbit model, external shocks evoked a polarization pattern and provided a basis for a virtual electrode induced phase singularity. These resulting reentrant scroll waves were underlie shock-induced arrhythmogenesis (Efimov et al., 1998).

There are some disadvantages of the isolated heart preparation. The isolated hearts do not metabolize the drugs; therefore, the potential effects of metabolites are not detected. This model may not be feasible to distinguish effects of drugs that are from the parent compound or from active metabolites. The drug binding to the tissue may be different in a protein-free buffer than in a protein-rich environment *in vivo*, therefore, it may eventually affect the drug concentration at the active site.

b. The methoxamine-sensitized rabbit model

The methoxamine-sensitized rabbit model was developed in the 1990s and has been used extensively (Carlsson et al., 1990; Akita et al., 2004; Kannankeril et al., 2010). Alpha-chloralose-anesthetized rabbits are pretreated with methoxamine, the α_1-agonist. These animals have an increased sensitivity to the repolarization-altering effects of some drugs, especially those that are relatively pure I_{Kr} blockers (e.g. dofetilide and dl-sotalol). Methoxamine predisposes the myocardium to reproducible TdP. These arrhythmias are the result of improper Ca^{2+} handling that leads to EADs and EAD-induced ectopic beats. This model demonstrated the importance of the STV_{QT} as a surrogate biomarker for proarrhythmic risk in the presence of QT prolonging drugs (Lengyel et al., 2008; Jacobson et al., 2011; Major et al., 2016). In this model, anesthetics may affect the electrophysiological properties of the heart including autonomic tone and cardiac ionic channel activity and may interfere with the electrophysiological effects of tested compounds (Carlsson, 2008; Inaba et al., 2011). Nevertheless, the methoxamine-sensitized rabbit remains a model of choice for

screening new drugs directly for their liability to induce TdP. Pro- and anti-arrhythmic properties of various drugs have been tested using this model (Diness et al., 2008; Carlsson et al., 2009; Jacobson et al., 2011; Khobragade et al., 2013; Mow et al., 2015; Varkevisser et al., 2015). Since the model depends on α–adrenoceptor sensitization, arrhythmogenic characteristics of drugs that concomitantly block I_{Kr} and α–adrenoceptors (e.g. quinidine, cisapride, quinolone) may not be fully appreciated by the model system (Carlsson, 2008).

There are important limitations of *in vivo* proarrhythmia models including the high variability in the occurrence of TdP. First, no model shows 100% reproducibility. Second, all the *in vivo* models utilize complex techniques suggesting it is difficult to induce TdP under experimental conditions. The rare occurrence of TdP arrhythmia in animal models suggests that other predisposing factors may contribute to TdP development. Third, the human heart has a repolarization reserve. It is likely that risk factors and/or genetic alterations in the target population reduce the repolarization reserve that leads to TdP. Fourth, the anesthetic protocol used in the rabbit *in vivo* pro-arrhythmia studies may have profound effects on the development of arrhythmias (Odening et al., 2008). In this regard, xylazine/ketamine-anesthesia would be recommended since it does not seem to affect repolarizing currents (Odening et al., 2008). Fifth, the more complex of *in vivo* proarrhythmia models may prove advantageous in that plasma protein binding and metabolites may be comparable to clinical studies.

c. **LQT transgenic rabbit models**

The transgenic rabbit models of LQT1 (KvLQT1-Y315S) and LQT2 (hERG-G628S) has been developed to mimic the human with QT prolongation, TdP and sudden cardiac death (Nerbonne, 2000; Brunner et al., 2008). It has been suggested that the APD prolongation and regional electrical heterogeneities propagated arrhythmia formation in LQT2 rabbits with a high rate of ventricular tachycardia/VF inducibility due to re-entry formation (Brunner et al., 2008).

In an LQT1-tachycardia-induced cardiac myopathy model, the promotors of VF were the increased APD dispersion, the steeper APD restitution and the spatial discordant alternans (Lau et al., 2015). Therefore, the risk for arrhythmia in LQTS underlies dynamic changes in which it was promoted by increased prolongation and dispersion of APD and the occurrence of discordant APD alternans.

In transgenic LQT2 rabbits, abnormalities in Ca^{2+} handling properties such as an increased spontaneous SR Ca^{2+} release via a phosphorylated, hyperactive RyR have been identified to promote EADs/arrhythmogenesis (Terentyev et al., 2014). There was also increased spatial dispersion of APD (Brunner et al., 2008). In addition, the VT and VF were easily inducible with left ventricular epicardial stimulation or developed spontaneously. The echocardiography and cardiac MRI studies have been shown to depict structurally and functionally normal hearts in transgenic LQT rabbits (i.e. LV ejection fraction, LV wall thickness, LV diameter and intraventricular septum thickness) (Brunner et al., 2008; Odening et al., 2013). Moreover, histological work-up of the heart tissue ruled out morphological changes such as myofibrillary disarray or fibrosis (Brunner et al., 2008). At the level of cardiomyocytes of LQT2 rabbit, the EADs developed easily upon reduction of K^+ concentrations and during sudden sympathetic surge whereas continuous perfusion with isoproterenol prevented EAD formation (Liu et al., 2012).

Recently, a transgenic rabbit model of LQT type 5 (LQT5) using a dominant-negative mutation in KVNE1 (KCNE1-G52R) was generated (Major et al., 2016). In contrast to LQT1 and LQT2 rabbits, these LQT5 transgenic animals exhibited only a very slightly prolonged QT interval whereas the STV_{QT} increased significantly.

d. MI model

The MI animal model is one of the most commonly used animal models to mimic human heart attack. Mortality after MI is relatively high and surviving patients are often severely compromised due to insufficient heart-pump function. At the cellular level, damages to the hearts contractile constituents are irreversible and the treatments are merely served to reduce symptoms. An anesthetized open-chest model with MI is useful for studying of the etiology of arrhythmia but not for assessing

the torsodogenic potential of NCEs (Eckardt et al., 1998). To generate MI, rabbits were anesthetized. The thorax was opened and the left anterior descending (LAD) artery was ligated. The MI is indicated by a light pallor of the myocardium below the ligature after suturing and a ST deviation on ECGs.

Another model recently use in MI studies is Watanabe heritable hyperlipidemic rabbits (WHHL)-MI rabbits. Compared with rodents, rabbits are better representative of human lipoproteins metabolism. For example, plasma cholesterol is distributed mainly in high-density lipoproteins (HDLs) in rodents rather than in low-density lipoproteins (LDLs) in both rabbits and humans. The aforementioned cholesteryl ester transfer protein (CETP) is naturally inactive in rodents, yet plays its key role in lipoprotein metabolism in both rabbits and humans. Another key player, liver apoB-editing protein, which edits apoB100 into apoB48, is just the opposite of CETP (Kobayashi et al., 2011). Even so, the rabbits are still resistant to atherosclerosis (AS). The WHHL rabbits are a very special strain originated in Japan which are naturally deficient in LDL-R and have hypercholesterolemia on chow diet and develop spontaneous AS (Buja et al., 1983). Selective breeding of WHHL rabbits obtains offspring with higher plasma cholesterol and accelerated AS not only in aorta but also in coronary arteries (designated as WHHL-CA rabbits). However, observations indicate that the mechanisms for MI in WHHL rabbits are different from those in humans (Shiomi et al., 2003). The incidence of MI in WHHL-CA rabbits was rather low (only 23%). Following further selective breeding of WHHL-CA rabbits, the incidence of MI could reach 97% (designated as WHHL-MI rabbits) (Shiomi et al., 2003).

CHAPTER III

MATERIALS AND METHODS

In order to test the hypotheses, this study was divided into 4 study parts as follow:

Study part 1: Characteristic of EMW in animal models of LQT and SQT

1. Approvals

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.

2. Surgical procedures

A total of 41 animals, distributed equally by sex, were used. All 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 via right internal carotid artery to determine 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.

3. Drugs

Dofetilide (Pfizer, Groton, CT, USA) was dissolved in 0.9% NaCl with the help of 0.1M 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, St. Louis, MO, USA) was dissolved in 0.9% NaCl. Pinacidil (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 5% ethanol. Analyses of dosing solutions were not performed; however, each drug was administrated to rabbits within 60 min of preparation.

4. Experimental protocol

Fig 5 illustrates the 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 0.1M HCl in normal saline (n=6), 10 μg/kg/min defetilide (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 (Kijtawornrat et al., 2006a; Kijtawornrat et al., 2006b; Kijtawornrat et al., 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.

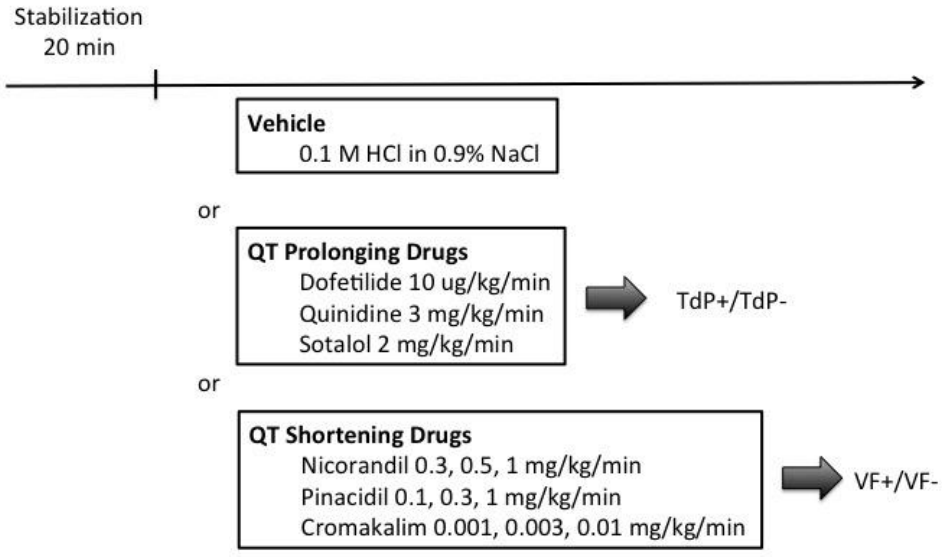


Figure X. Panel shows an overview of the experimental protocol for testing electro-mechanical window (EMW) in QT prolonging drugs and QT shortening drugs.

5. 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, VA, USA). All ECG and LVP parameters were measure 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 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 HR by dividing the QT interval by the cube root of the preceding RR interval (Fridericia, 1920). EMW was calculated by EMW = QLVPend-QT. STV_{QT} was calculated using the formula: $STV_{QT} = \sum \left| Dn+1-Dn \right| /[30x\sqrt{2}]$ whereas D = the duration of QT (ms) without the occurred VPCs (Thomsen et al., 2004). All measurements were performed during periods without abnormal beats. End-diastolic pressure (EDP) and end-systolic pressure (ESP) were traced from the LVP tracing. dP/dt_{max} and dP/dt_{min} were obtained from the LVP. Percent changes from baseline were calculated for each drug dose. Data are presented as mean ± standard error of mean (SEM). In LQT2 model, comparisons between vehicle and test compounds were made using two-way analysis of variance (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.

Study Part 2: Use of EMW for predicting ischemia-induced ventricular fibrillation in rabbit with LAD ligation

1. Approvals

This study was approved by the Institutional Animal Care and Use Committee of Chulalongkorn University (protocol number 1673033). All experimental animal procedures were performed in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals (2011).

2. Animals

A total of eighteen healthy mature New Zealand White rabbits, weighing between 2-3 kg of either gender were purchased from Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University (Bangkok, Thailand). They were housed as group from the time of arrival until the end of the study in a rabbit caging system maintained at a temperature of 21±2 °C, a relative

humidity of 50-70%, and a 12:12 hr light:dark cycle. All animals were received commercial diet and water ad libitum.

3. Experimental procedures

All rabbits were given tiletamine/zolazepam (Zoletil, Virbac, France) 25 mg/kg intramuscularly. After induction, the hair over the thoracic and the neck area was clipped. The rabbit was secured in a dorsal recumbency on water-circulating heating pad to maintain body temperature. The propofol (8-30 mg/kg/hr) was administered intravenously to achieve an appropriate anesthetic plane.

A lead I ECG was obtained by placing electrodes between the right (-) and left (+) front legs. The ground electrode was placed at right hind limb. Cut down was performed to isolate the right carotid artery. A high-fidelity micromanometer catheter (Millar Instruments, Texas, USA) was placed retrograde in the left ventricle via right common carotid artery for recording the LVP.

The process of ischemia-induced arrhythmia been described previously (Brown et al., 2014). In brief, the thorax was opened via a midline sternotomy. After the pericardium was opened, the 4-0 monofilament polypropylene sutures with needle were placed around the LAD coronary artery and a descending branch of the left circumflex artery (Zhao et al., 2008). The rabbit was allowed to stabilize for at least 20 minutes. Thereafter, the animal was subjected to an acute, ischemic insult for 30 minutes by tightening of the coronary arteries on polyethylene tube, PE-150. MI was visually confirmed by color (i.e. cyanotic) changes in distal distributions of the ligation, and the ST elevation was observed using ECG. During the occlusion period, the experiment was stopped as soon as VF started, and rabbits were euthanized with 150 mg/kg pentobarbital sodium (Nembutal, Ceva Sante Animale, Libourne, France) administered intravenously. In case of no VF occurred during occlusion, rabbits were monitored until the end of 30 min occlusion period. At the end of the occlusion period, all rabbits were euthanized while they were under general anesthesia. Rabbits with or without VF during ischemia were allocated into the VF+ and VF- groups respectively. ECG and LVP were recorded from the beginning of the experiment and continue to monitor until the end of experiment. All parameters were obtained at

baseline and a minute before VF development (VF+) or at 30-min after coronary ligation (VF-). Fig. 6 illustrates the experimental protocol.

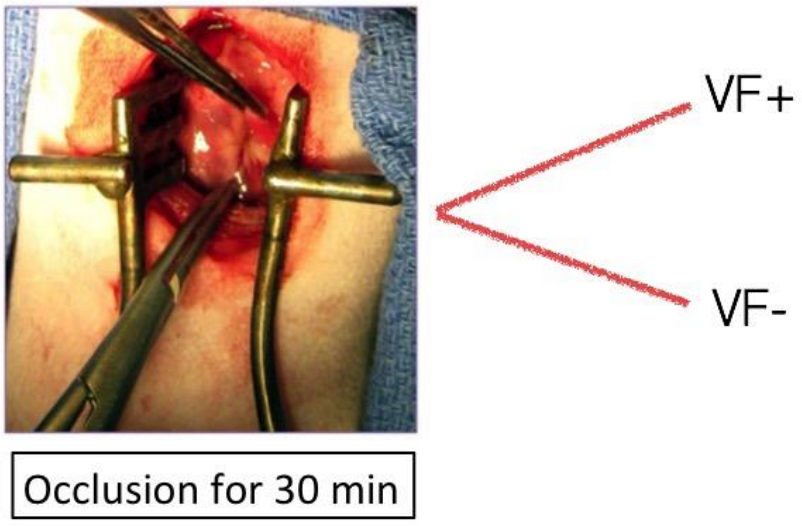


Figure 6. The 4/0 polypropylene suture was ligated around the left anterior descending (LAD) coronary artery and a descending branch of the left circumflex artery for 30 minutes. The rabbits were divided into 2 groups depend on ventricular fibrillation occurrence during occlusion period.

4. Data analysis

Data were recorded and analyzed using Acq 3.9.1 acquisition systems (Biopac MP150, Santa Barbara, CA, USA). Standard ECG (RR, PQ, QRS, QT) and LVP (EDP, ESP, dP/dt_{max}, dP/dt_{min}, tau) parameters, and the EMW were manually measured. The values of the parameters in both groups of rabbits, i.e. those groups with and without

VF, were averaged from baseline, a minute just before the VF occurred, or at the end of 30th minute of occlusion. An average of the 31 cardiac cycles per timepoint originated from the sinus node was reported. The QT was defined as the duration from the beginning of the Q wave to the end of T wave. QTcF was calculated according to Fridericia's equation (Fridericia, 1920). STV_{QT} was calculated using the formula: $STV_{QT} = \sum \left| Dn+1-Dn \right| /[30x\sqrt{2}]$ whereas D = the duration of QT (ms) without the occurred VPCs (Thomsen et al., 2004). The EMW was calculated using the following formula: EMW = QLVPend-QT (van der Linde et al., 2010b). The tau was calculated according to Glantz method (Raff and Glantz, 1981). The VF is characterized by ventricular complexes that are changing in frequency and amplitude in the ECG, resulting in the irregular patterns of ventricular excitation (Moe and Abildskov, 1964; Witkowski and Penkoske, 1990). The ventricular premature complex (VPC), R-on-T and VF were defined according to the Lambert conventions (Walker et al., 1988). The frequency of arrhythmic beats (i.e. VPC and R-on-T) that developed during occlusion was also reported.

Statistical analyses were performed with commercially available software. All values were expressed as means ± SEM. The differences in incidences of VF were compared using Fisher's exact test. Values for ECG and LVP parameters were compared between groups (i.e. VF- versus VF+) and between timepoints (i.e. baseline vs occlusion) two-way ANOVA with repeated measures followed by Tukey post-hoc analyses. Frequencies of arrhythmic beats at occlusion period were compared between groups using *t*-test. Statistical significance was achieved when $p < 0.05$. Receiver operating characteristic (ROC) curve analysis was performed to determine the predictive power of EMW, STV_{QT}, QT and QTcF. 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 VF. The optimal cut-off values were determined using Youden indexes.

Study Part 3: Characteristic of EMW in the rabbit model of dofetilide-induced TdP

1. Approvals

The approval has been described in detail previously in study part 1.

2. Animals

Six healthy mature male New Zealand White rabbits were purchased from Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University (Bangkok, Thailand). The animal care has been described in detail previously in study part 1.

3. Drug preparation

Dofetilide (Tikosyn, Pfizer, NY, USA) was prepared as a solution by 0.9% NaCl and acidified with 0.1 M hydrochloric acid. Verapamil hydrochloride (Hospira, Pfizer, IL, USA) was dissolved in 0.9% NaCl

4. Experimental procedures

Anesthesia and instrumentation were performed as described earlier in study part 1.

After instrumentation, rabbits were allowed to stabilize for at least 10 min. Then ECG and LVP were obtained. After all data were collected, verapamil (0.3 mg/kg) or vehicle (0.9% NaCl) was started and continued for 10 min. After the end of verapamil or vehicle infusion, rabbits were given intravenously with dofetilide 30 μg/kg/min for 15 min or until the occurrence of TdP. All rabbits were euthanized at

the end of experiment while they were under general anesthesia with sodium pentobarbital (120 mg/kg; Nembutal, Ceva Sante Animale, Libourne, France) in accordance with American Veterinary Medical Association guidelines. Fig. 7 illustrates the experimental protocol.

Figure 7. Panel shows an overview of the experimental protocol for testing electro-mechanical window (EMW) in verapamil prevented dofetilide-induced TdP

5. Data analysis

ECG and LVP were analyzed for heart rate, standard ECG intervals, EMW, and STV_{QT} at baseline, the last minute of verapamil dosing, and post dofetilide dosing by using ECG Auto Software (EMKA Technologies, Falls Church, VA, USA). The QTcF, EMW and STV_{QT} were calculated as described earlier. The dP/dt_{max}, dP/dt_{min} , contraction time (ctrT), and relaxation time (relT) were obtained from the LVP.

Statistical analyses were performed with commercially available software. Data are presented as mean ± SEM. All data points were averaged from 60 s of recording. Differences among each time points were determined using one-way ANOVA with repeated measured design or paired *t*-test if applicable. When indicated by a

significant, specific means were compared by Tukey test. A probability value of $P < 0.05$ was considered to be significant.

Study Part 4: Effects of preload, contractility, blood pressure, and heart rate on EMW in anesthetized rabbits

1. Approvals

The approval was the same protocol number as in study part 1.

2. Animals

A total of twenty-two male New Zealand White rabbits (*Oryctolagus cuniculus*) supplied by Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University (Bangkok, Thailand) were used. All rabbits were weighed between 2 and 2.8 kg. A full description of the animal care is given in study part 1.

3. Experimental procedures

The full description of anesthesia and instrumentation are given in study part 1.

In order to determine effects of preload on EMW, rabbits (n=5) were received 0.9% NaCl infusion at escalating volumes (0.5%, 1%, 1.5% of body weight). Each volume was administered for 15 min.

To examine the effects of HR on EMW, the rabbits (n=5) were intravenously administered escalating doses of cilobradine (2.5, 5 and 10 mg/kg/15min). Cilobradine was given as a bolus to reduce heart rate according to previous publication (Kruger et al., 2000).

In order to determine effects of arterial pressure on EMW, rabbits (n=8) were received escalating concentrations of phenylephrine (3, 5, 10 μg/kg/min) for 15 minutes. Then hemodynamics was allowed to return to baseline. After that, rabbits

were given escalating doses of sodium nitroprusside (1, 3, 5 μg/kg/min) for 15 minutes. Doses of phenylephrine and sodium nitroprusside were selected according to previous publications (Farkas et al., 2010; Westphal et al., 2010).

In order to determine effects of contractility on EMW, rabbits (n=4) were received intravenous infusion of esmolol at a rate of 0.5 mg/kg/min for 15 minutes. After that the hemodynamic and inotropic states were allowed to return to baseline. Rabbits were received intravenous infusion with milrione at a rate of 1 μg/kg/min for 15 minutes. Doses of both esmolol and milrinone were chosen according to previous publications (Uchida et al., 2005; Lange et al., 2006). Fig. 8 illustrates the experimental protocol.

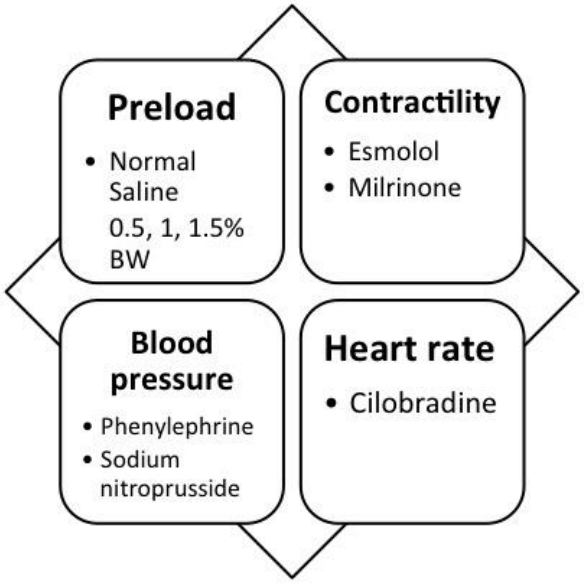


Figure 8. One of the study factors (preload, contractility, blood pressure or heart rate) was randomly applied to anesthetized rabbit.

ECG and LVP were recorded throughout the study. At the end of each experiment, rabbits were euthanized while they were under general anesthesia with sodium pentobarbital (120 mg/kg, intravenously) (Nembutal, Ceva Sante Animale,

Libourne, France) in accordance with American Veterinary Medical Association guidelines 2013.

4. Drugs

Cilobradine (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 0.9% NaCl. Esmolol (Brevibloc, Baxter Healthcare Corporation, Deerfield, IL, USA), milrinone (Primacor, Sanofi, Paris, France), phenylephrine hydrochloride (West-Ward, Eatontown, NJ, USA) and sodium nitroprusside (Nitropress, Pfizer, NY, USA) were diluted in 0.9% NaCl.

5. Data analysis

ECG was analyzed for HR, intervals and STV_{QT} while LVP was analyzed for EDP, ESP, dP/dt_{max}, dP/dt_{min} and tau. Systolic blood pressure (SBP) was estimated from the systolic LVP. Data were analyzed at baseline (before drug infusion) and at the end of each drug or 0.9% NaCl infusion using ECG Auto Software (EMKA Technologies, Falls Church, VA, USA). 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, QTcF, EMW and STV were measured as described earlier in study part 1.

Statistical analyses were performed with commercially available software. All values were expressed as means ± SEM. The differences among escalating doses of drugs from baseline were evaluated by Student's *t*-test or 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 among EMW to HR, SBP and contractility. In all cases, $p < 0.05$ was considered as statistically significant.

CHAPTER IV
RESULTS

The results of this study were organized into 4 study parts as follow:

Study part 1: Characteristic of EMW and STV_{QT} in animal models of LQT and SQT

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 HR were ranging from 178 to 288 bpm, the PQ intervals were 63.3 ± 1.43 ms, the QRS complexes were 50.6 ± 1.22 ms, the QT intervals were 163 ± 3.35 ms, the QTcF intervals were 257 ± 3.75 ms, the STV_{QT} were 2.33 ± 0.319 ms and the EMW were 21.8 ± 1.82 ms (Fig 9).

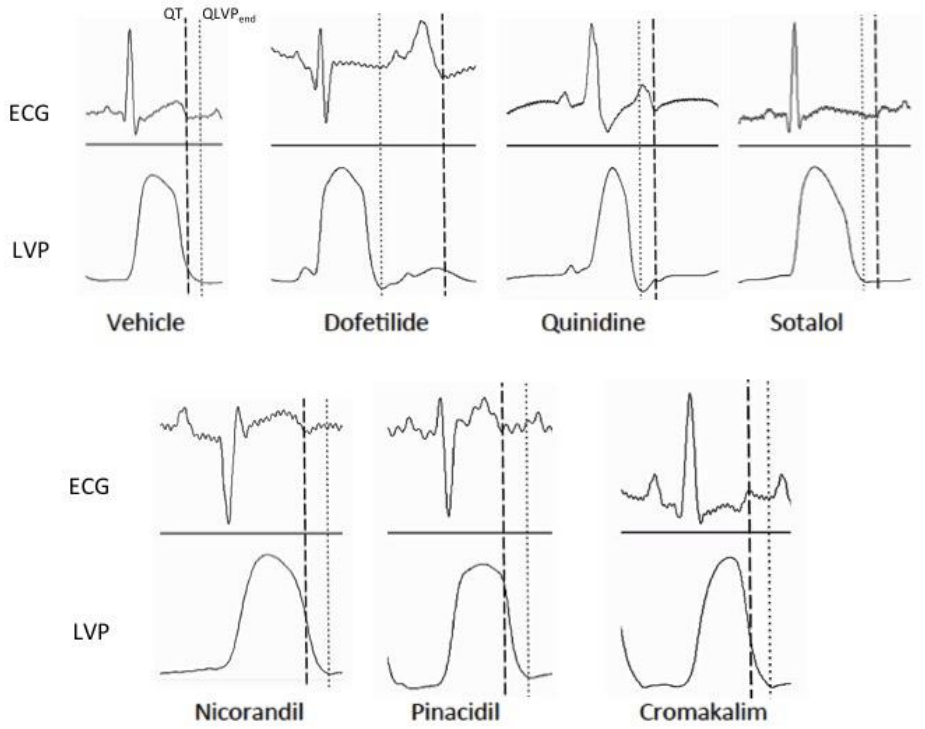
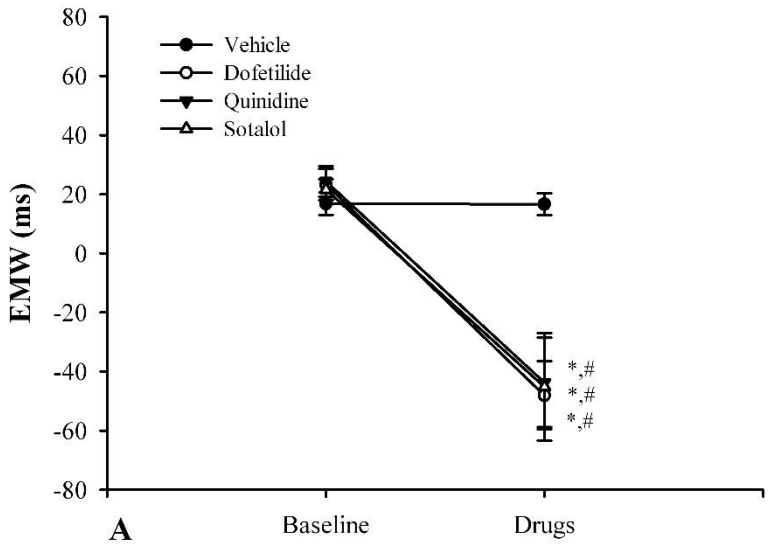


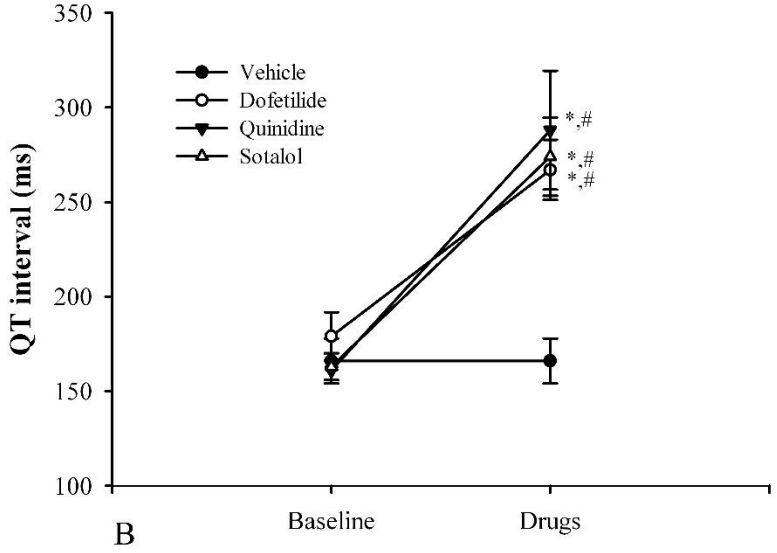
Figure 9. Examples of bipolar, transthoracic electrocardiograms (ECG) and left ventricular pressure in anesthetized rabbits receiving vehicle (n=6), dofetilide (n=6), quinidine (n=6), sotalol (n=6), nicorandil (n=7), pinacidil (n=5) and cromakalim (n=5). The duration between the dot line (QLVPend) and the dash line (QT interval) is the EMW. Notice the abbreviation of EMW to negative values when the rabbits received QT prolonging drugs whereas it is more positive in response to QT shortening drugs.

A. Effects of known QT lengthening compounds on ECG and LVP parameters

Effects of agents known to lengthen QTc interval were shown as percent changes form 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. 10A.), QT (Fig. 10B.) and QTcF (Fig. 10C.) versus time points (before and after drug administration) were shown 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. STV_{QT} remained unchanged at dosage of QT prolong agents (Table 2, Fig. 10D). 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 all of QT prolonging drugs.




350
300
250
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QT interval (ms)
Vehicle
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Baseline
Drugs
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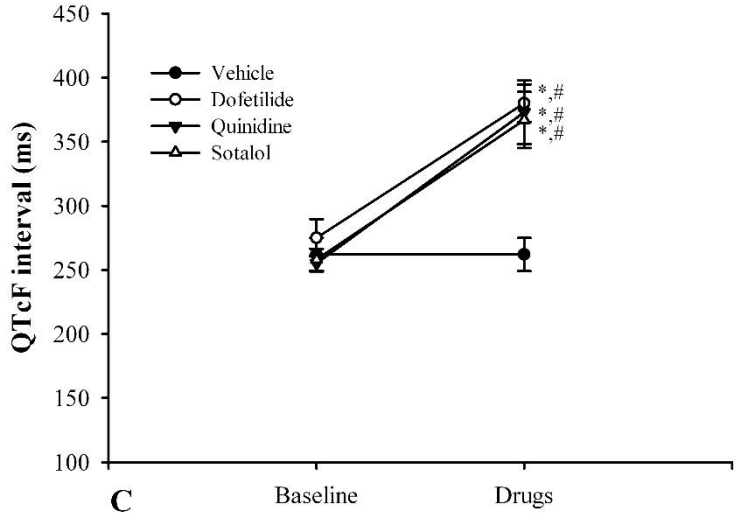
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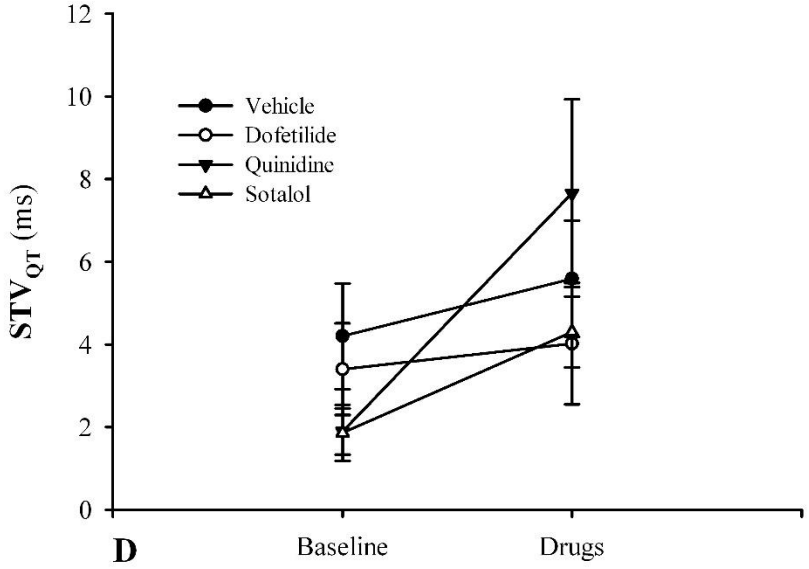


Figure 10. Plots of mean and standard error of mean for electromechanical window (EMW, A), QT interval (B), QTcF interval (C) and short-term variability of QT interval (STV_{QT}, D) versus timepoints (before and after drug administration). Rabbits were exposed to vehicle (0.1M HCl in 0.9% NaCl; n=6) or test articles known to lengthen QTcF interval (dofetilide; n=6, quinidine; n=6, and sotalol; n=6). Each data point is the average of cardiac cycles for 1 minute. Doses of the reference compounds were dofetilide (10 μg/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.

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

	Baseline				Treatment			
	Vehicle n=6	Dofetilide n=6	Quinidine n=6	Sotalol n=6	Vehicle	Dofetilide	Quinidine	Sotalol
ECG Parameters								
RR (ms)	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 (ms)	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 (ms)	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 (ms)	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 (ms)	262±12.9	275±14.6	255±6.2	258±8.5	262±13	380±14.6*	373±24.8*	367±21.9*
STV_{QT} (ms)	4.2±1.3	3.4±1.1	1.9±0.55	1.9±0.7	5.6±1.4	4±1.5	7.7±2.3	4.3±0.9
EMW (ms)	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/s)	2895±454	2965±397	2852±201	2924±308	2902±347	3181±315	1107±298*	1691±154*
dP/dt_{min} (mmHg/s)	- 2251±358	- 2424±186	- 2665±219	- 2376±277	- 2279±309	-2424±133	- 1209±202*	- 1492±159*
relT (ms)	50.2±1.7	58±4	51±1.4	53.1±1.4	50.2±1.7	65.7±4.6*	52.9±1.3	57.2±1.5

Data are presented as mean ± SEM. *compared effects of drug administration to its baseline value whereas #compared effects of drug administration among groups to vehicle group, statistical difference ($P<0.05$). QTcF, the corrected QT interval by Fridericia's formula; STV_{QT}; short-term variability of QT interval,

EMW, electromechaincal window; EDP, end-diastolic pressure; ESP, end-systolic pressure; relT, relaxation time

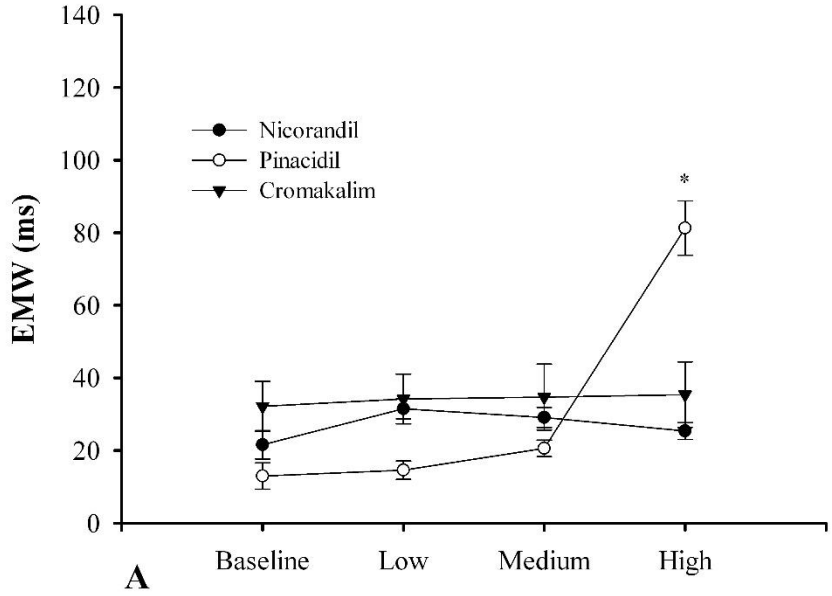
B. Effects of known QT shortening compounds on ECG and LVP parameters

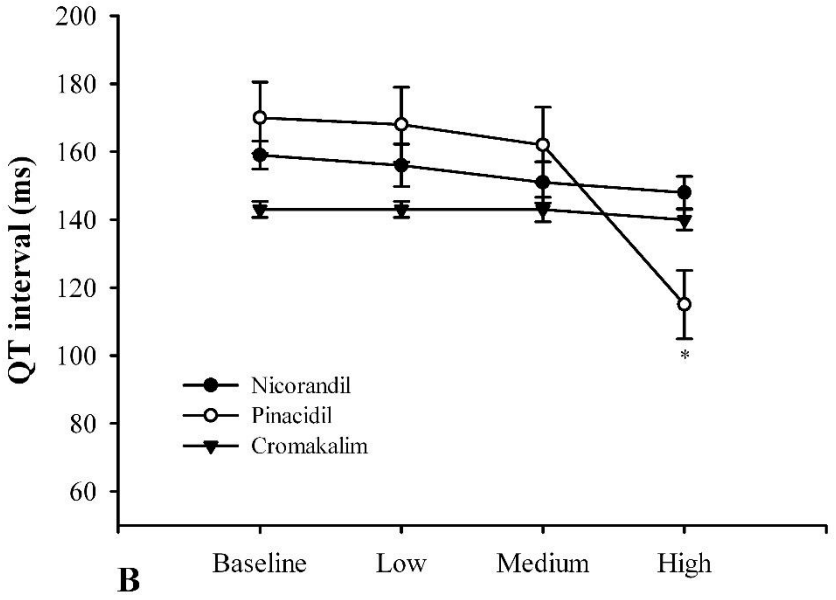
Effects of agents known to shorten QTc interval were shown as percent changes from baseline in Table 2. The durations of RR, PQ and QRS were not affected by all three doses of nicorandil, pinacidil and cromakalim. Plots of EMW (Fig. 11A.), QT (Fig. 11B.) and QTcF (Fig. 11C.) versus doses of QT shortening drugs were shown. Only pinacidil markedly lengthened EMW and shortened QT and QTcF intervals when compared to those values obtained at baseline ($p<0.05$). Furthermore, STV_{QT} after 1 mg/kg/min pinacidil was significantly larger than after other doses (Table 3, Figure 11D). All measured parameters of LVP (Table 11) 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 compared with baseline.

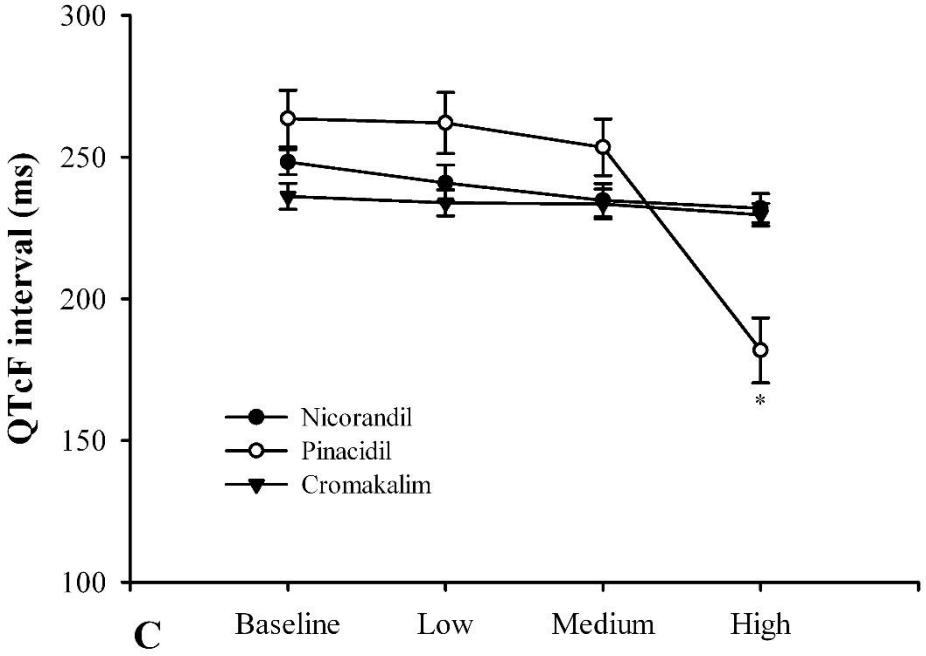
Table 3. Effects of agents known to shorten QTc interval on electrocardiograms (ECG) and left ventricular pressure (LVP) parameters.

	Baseline			Treatment (Dose 1 - Low)			Treatment (Dose 2 - Medium)			Treatment (Dose 3 - High)		
	Nicorandil n=7	Pinacidil n=5	Cromakalim n=5	Nicorandil	Pinacidil	Cromakalim	Nicorandil	Pinacidil	Cromakalim	Nicorandil	Pinacidil	Cromakalim
ECG Parameters												
RR (ms)	262±10.4	266±19.3	222±6.1	270±16.1	262±19.7	227±8.2	267±18.9	261±2.5	229±9.1	259±17.2	256±24.4	226±9.2
PQ (ms)	65.2±3.6	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	62.5±1.9
QRS (ms)	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	56.8±5
QT (ms)	159±4.1	170±10.5	143±2.3	156±6.3	168±11	143±2.4	151±6.1	162±1.1	143±3.6	148±4.7	115±10.1*	140±3
QTcF (ms)	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*	230±4
STV_{QT} (ms)	1.9±0.4	1±0.4	1.9±0.5	2.2±0.6	1.3±0.2	1.9±0.5	3.2±1	1.6±0.5	1.6±0.4	1.6±0.7	3.4±0.4*	1.8±0.4
EMW (ms)	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*	35.4±9
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	-0.7±0.7
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*	57.3±5.6
dP/dt $_{max}$ (mmHg/s)	2577±383	3606±299	3269±413	1972±341	2779±196*	3177±486	1769±357	2174±78.5*	3135±581	1938±288	1444±68.4*	2707±501
dP/dt $_{min}$ (mmHg/s)	-2411±302	-2725±218	-2929±331	-1466±133	-1921±242*	-2700±266	-1274±91.2	-1514±158*	-2507±233	-1458±92.3	-1196±126*	-2088±243
relT (ms)	50.7±0.7	52.7±1.6	54.2±2.6	50.1±1.6	52±2.5	56.3±3.7	46.7±1.2*	49.5±1.7	57±4.2	46.1±0.7*	53±1.4	57±4.5

Data are presented as mean ± SEM. *compared effects of drug administration to its baseline value, statistical difference ($P<0.05$). QTcF, the corrected QT interval by Fridericia's formula; STV_{QT}; short-term variability of QT interval, EMW, electromechaincal window; EDP, end-diastolic pressure; ESP, end-systolic pressure; relT, relaxation time.


140
120
100
80
60
40
20
0
EMW (ms)
Nicorandil
Pinacidil
Cromakalim
*
Baseline
Low
Medium
High
A


200
180
160
140
120
100
80
60
QT interval (ms)
Nicorandil
Pinacidil
Cromakalim
*
Baseline
Low
Medium
High
B



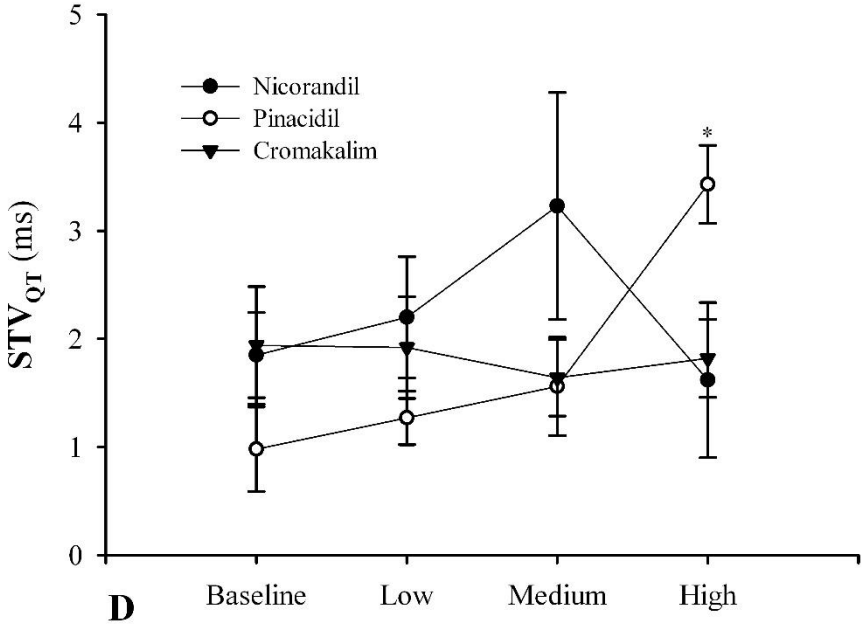
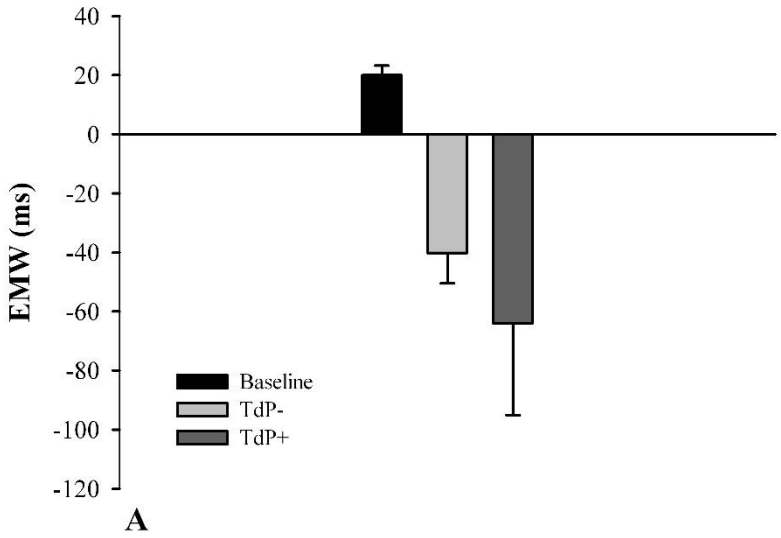


Figure 11. Plots of mean and standard error of mean for electromechanical window (EMW, A), QT interval (B), QTcF interval (C) and short-term variability of QT interval (STV_{QT}, D) versus doses of QT shortening drugs. Rabbits were exposed to escalating concentrations of test articles known to shorten QT interval (nicorandil; n=7, pinacidil;

n=5, and cromakalim; n=5). Each data point is the average of cardiac cycles for 1 minute. 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.

C. 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. To investigate whether the EMW differed between TdP positive experiments versus arrhythmia negative experiments, data of all experiments were pooled as shown in Figure 12. Compared to baseline (20±3.27 ms) all the QT prolong drugs decreased the EMW. In experiments where TdP did occur no significant difference were observed compared to conditions where no TdP could be triggered (-64±31.1 ms versus -40.2±10.3 ms). However, the EMW tend to more negative in TdP-occur rabbits. Noted that EMW and STV_{QT} in VF incidence was not analyze because only one rabbit was in VF condition.



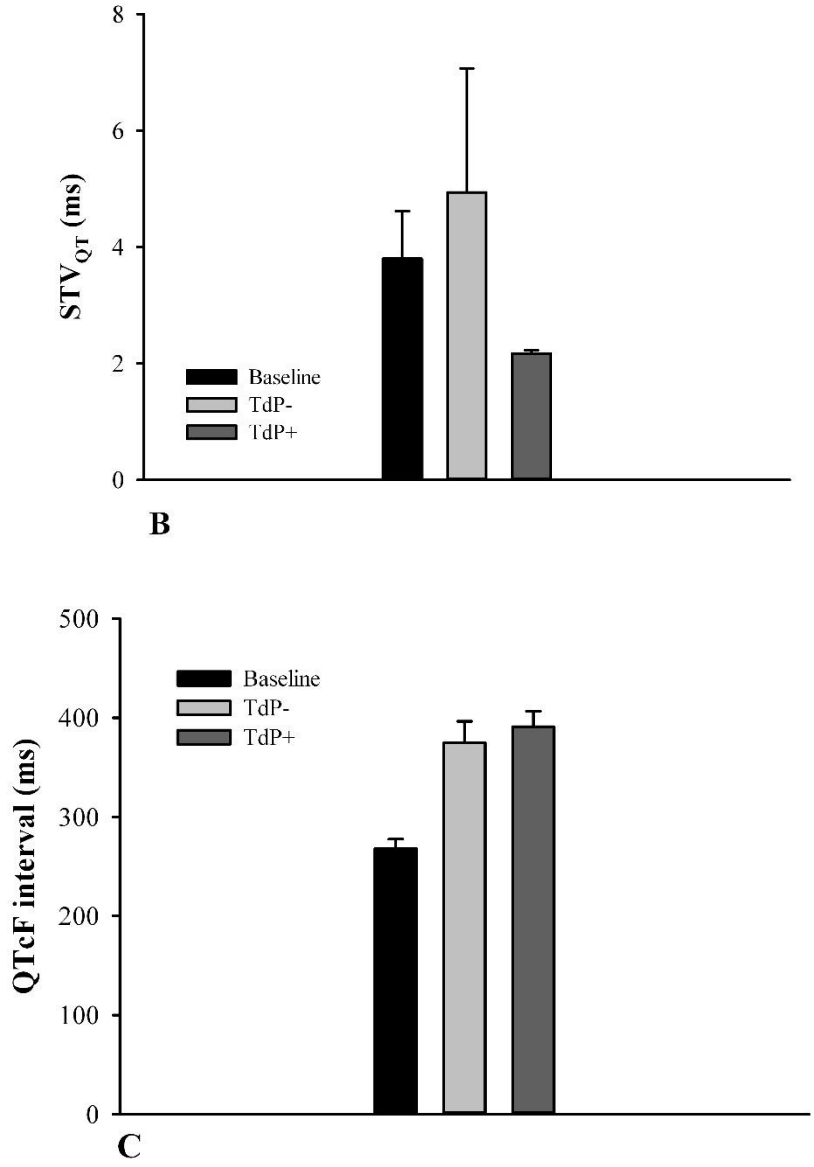


Figure 12. Bar charts illustrating the electro-mechanical window (EMW; A), short-term variability of QT interval (STV_{QT}; B) and QTcF interval (C) at baseline and dofetilide administration. Data of all experiments (vehicle and dofetilide, n=12) were pooled and classified according to the occurrence of TdP. In experiments where TdP did occur (TdP+), EMW tend to have more negative value compared to conditions where no TdP occur (TdP-). Bars represented mean±SEM.

D. 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) was shown in Fig 13. The relationship between EMW and QT interval was EMW = 118 – (0.6*QT), having and r^2 of 0.80. This demonstrates that the relationship between the two is highly significant ($p<0.001$).

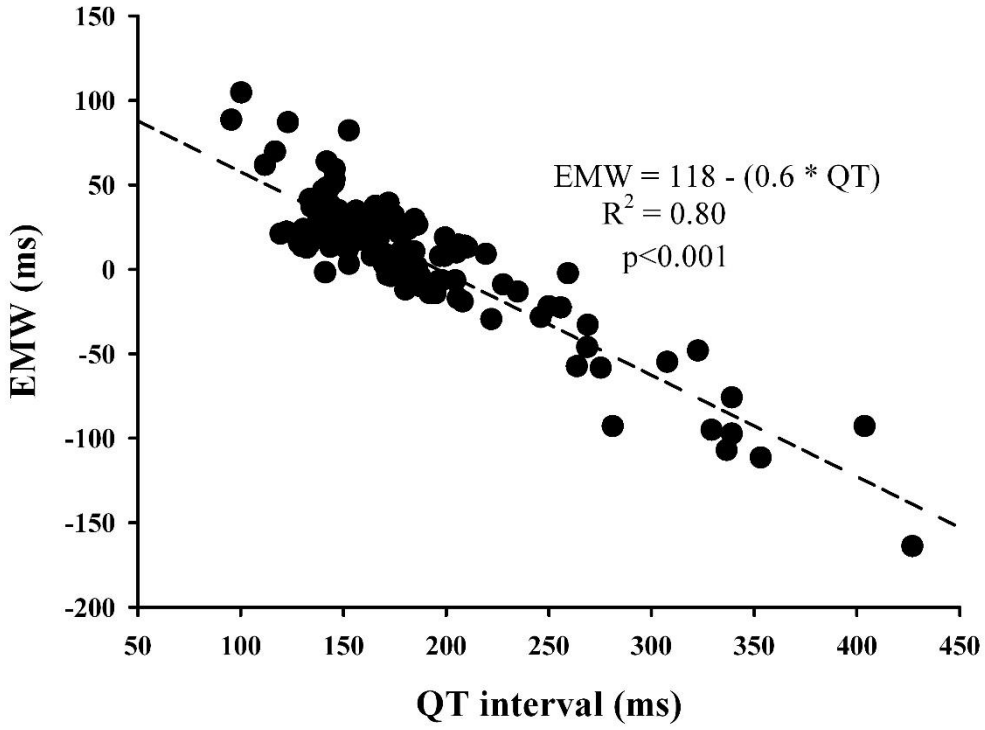


Figure 13. Plot of averages of electromechanical window (EMW) versus QT interval for 41 anesthetized rabbits that received either vehicle (0.1M HCl in 0.9% NaCl) or drugs known to lengthen QT intervals (i.e. dofetilide, quinidine and sotalol) or known to shorten QT intervals (i.e. nicorandil, pinacidil and cromakalim). Notice that the relationship between EMW and QT interval was very high.

E. 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 was demonstrated in Fig 14. The 25th percentiles for vehicle, QT prolonging drugs and QT shortening drugs were 15.9, -75.8 and 26.3 ms while the 75th percentiles were 32.1, -19.1 and 65.7 ms, respectively. The medians for those groups indicated by horizontal lines within the boxes were 23.6, -31.2 and 35.3 ms, respectively. There were no outliers in the data. There was statistically significant difference in the mean values of EMW among the groups (baseline versus QT prolonging drugs, $p<0.001$; baseline versus QT shortening drugs, $p<0.05$).

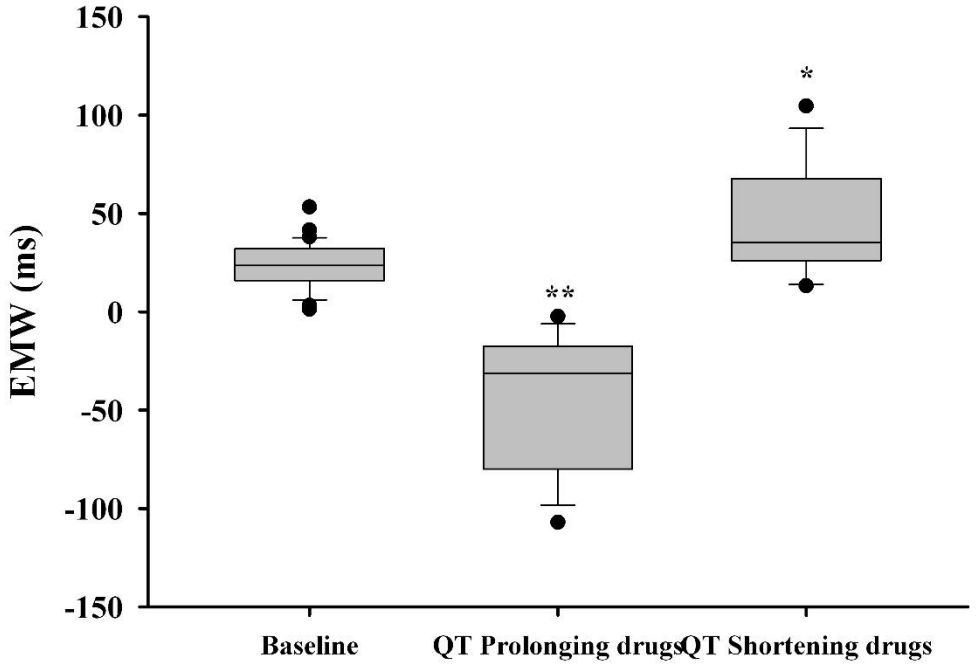


Figure 14. A box-and-whisker plot showing the distribution of electromechanical window (EMW) values for baseline, QT prolonging drugs and QT shortening drugs, n=41. The boxes represent the 25th to 75th percentiles, and horizontal lines within the boxes indicate the medians. An asterisk (*) indicates $p < 0.05$ and (**) indicates $p<0.001$ compared with baseline data.

Study Part 2: Use of EMW for predicting ischemia-induced VF in rabbit with LAD ligation

A. Incidence of VF

Readable ECG and LVP data were obtained from all rabbits. After ligation of LAD and a descending branch of the left circumflex arteries, VF developed during the occlusion period in ten out of 18 rabbits (55.6 %, $p<0.01$). The ST elevation was observed after occluded the coronary arteries in all rabbits. A representative tracing of the ECG (lead I) and of the LVP signals at baseline, during the development of VF (VF+) and during occlusion (VF-) in anesthetized rabbits were shown in Fig 15.

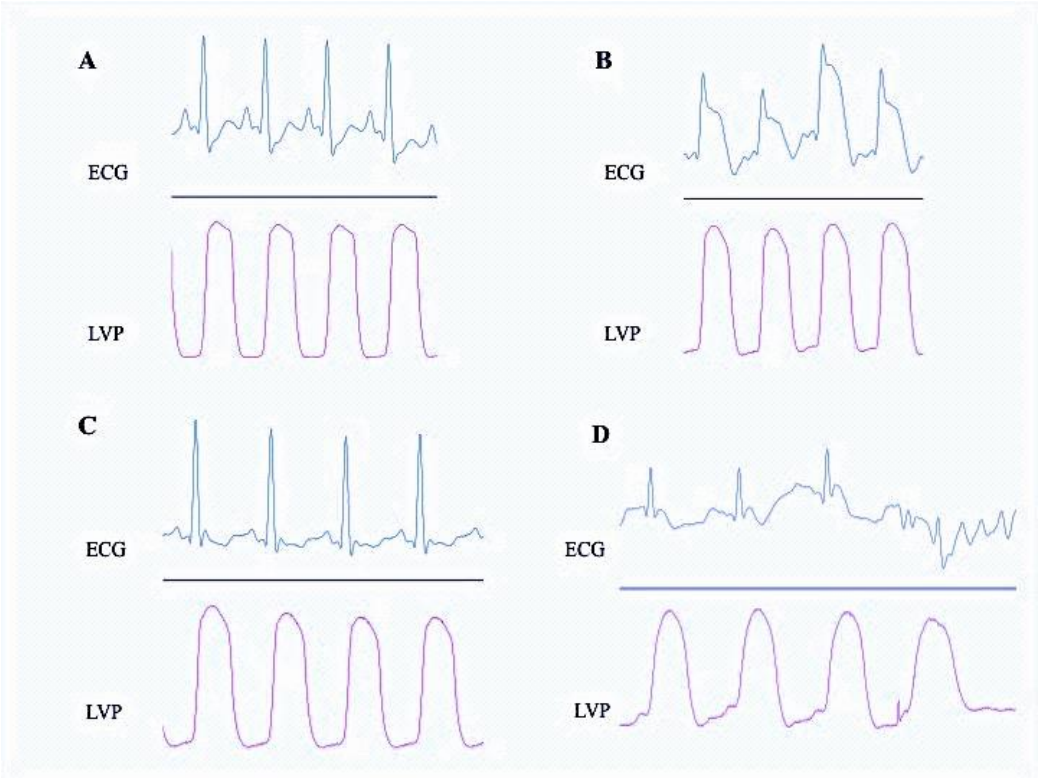
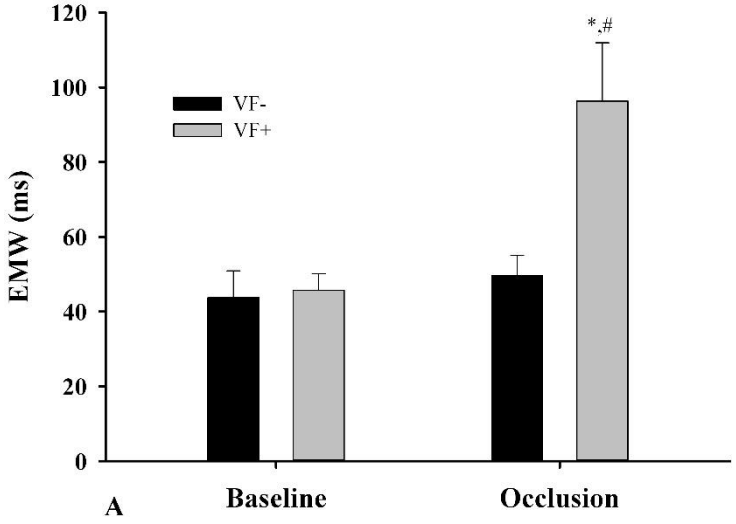


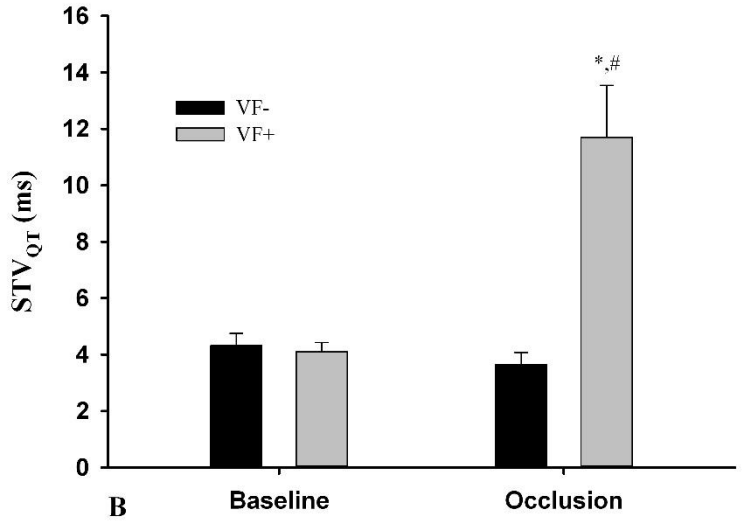
Figure 15. Representatives of electrocardiogram (ECG) tracings (top) and left ventricular pressures (LVP) (bottom) obtained from anesthetized rabbits during baseline (A, C) and occlusion (B, D) periods. A = the ECG and LVP waveforms observed during the baseline in rabbit without VF, n=8. B = the ECG and LVP waveforms observed during the occlusion period in rabbit without VF. C = the ECG and LVP waveforms observed during the baseline in rabbit with VF, n=10. D = the ECG and LVP waveforms observed during the occlusion period in rabbit with VF notice the development of VF.

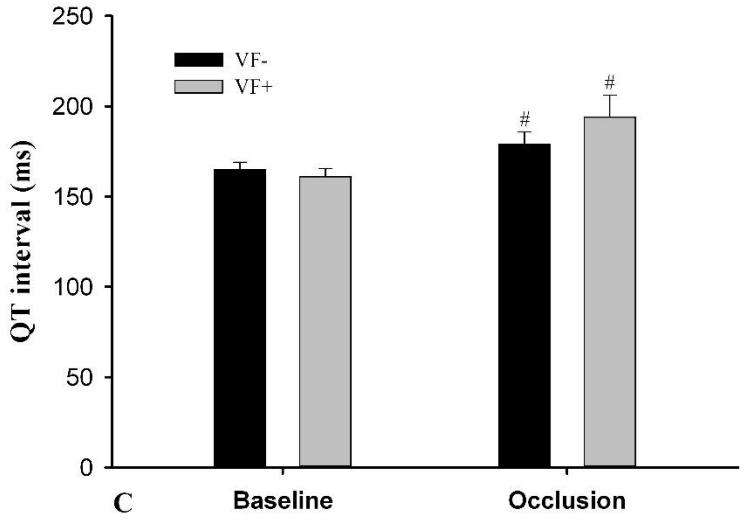
B. Changes of EMW, STV_{QT} and parameters of ECG and LVP

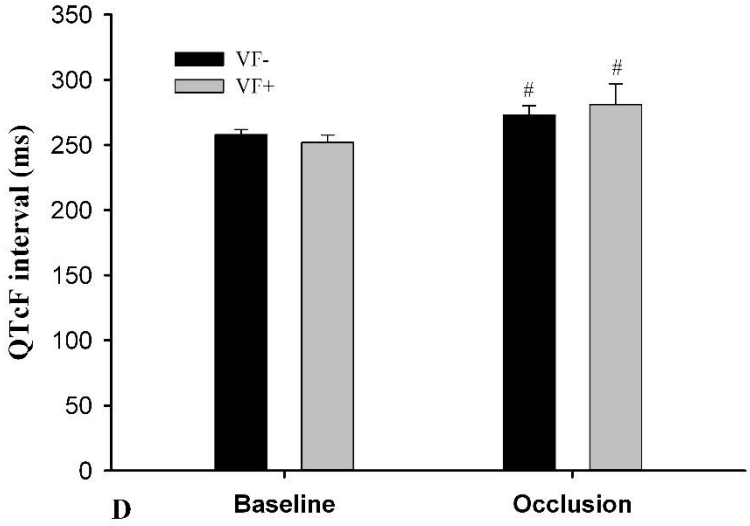
At baseline, all parameters did not differ between VF+ and VF- rabbits (Fig. 16). The data of VF- obtained at the end of occlusion period were used to compare with the data obtained from the VF+ rabbits obtained a minute just before the development of VF. In VF+ rabbits, the EMW at occlusion (96.3±15.6 ms) was significantly higher than baseline value (45.7 ± 4.4 ms) and value of VF- rabbits at occlusion (49.5±5.6 ms) ($p<0.05$, Fig. 16A). The STV_{QT} (Fig. 16B) in VF+ rabbits at occlusion (11.7±1.8 ms) was significantly greater than the STV_{QT} at baseline (4.1 ± 0.3

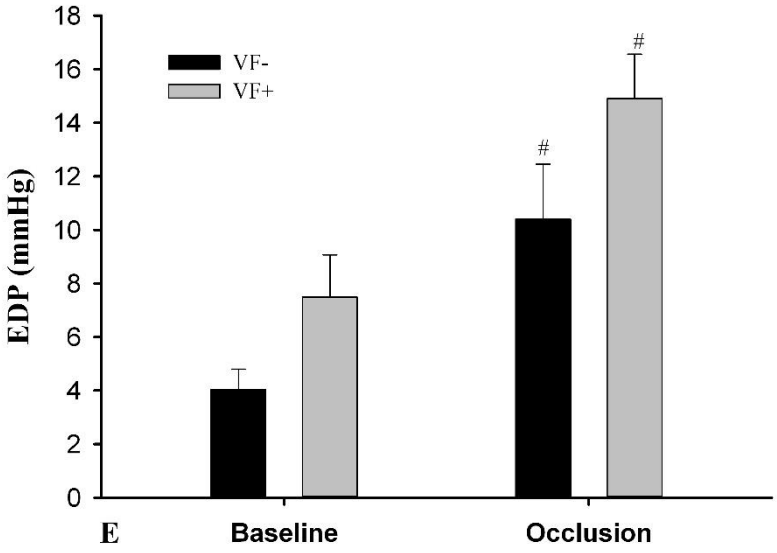
ms) and that value in VF- rabbits obtained during occlusion (3.7±0.4 ms) ($p<0.05$). During occlusion, the QT interval (Fig. 16C), QTcF interval (Fig. 16D), EDP (Fig. 16E) and dP/dt_{min} (Fig. 16F) were significantly increased from baseline in both groups ($p<0.05$) but these parameters did not differ between groups. During occlusion, the ESP (Fig. 16G) and dP/dt_{max} (Fig. 16H) were significantly decreased from baseline in both groups ($P<0.05$) but these values were not differed between groups at occlusion. In VF+ rabbits during occlusion, the tau was significantly increased when compared with data obtained at baseline of the same group and at occlusion of the VF- rabbits ($p<0.05$) (Fig. 16I). In VF- rabbits at occlusion, tau was also significantly higher than tau at baseline of the same group ($p<0.05$). During occlusion, the heart rate (HR) of VF+ rabbits were significantly lower than the HR of VF- rabbits (HR: baseline 236±7.1 bpm and 235±11 bpm, respectively, and during occlusion 187±7.4 bpm and 221±9.5 bpm, $p<0.05$, respectively).

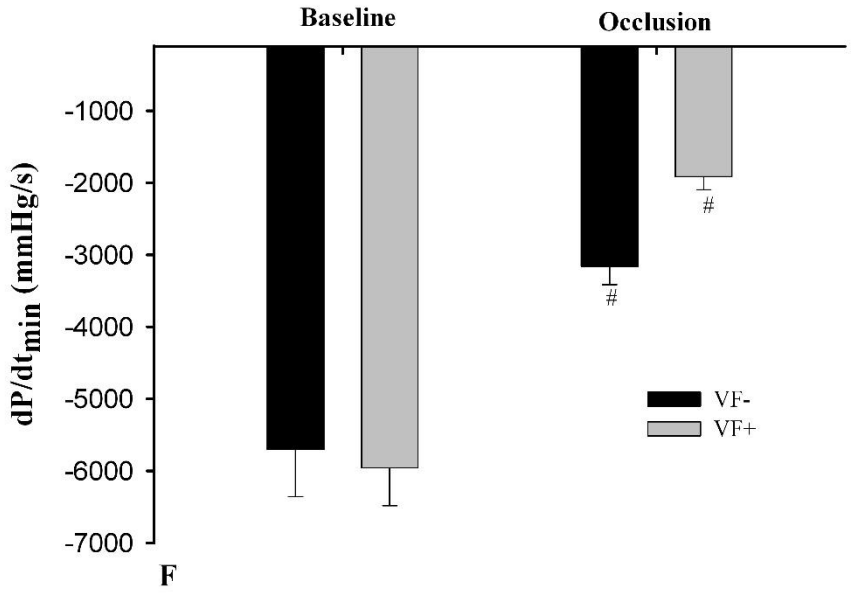


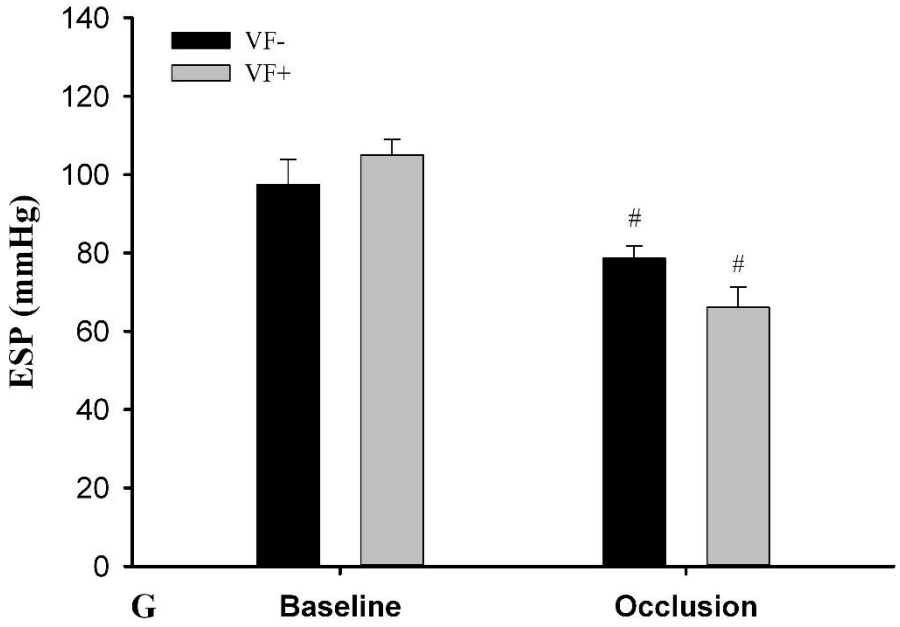

16
14
12
10
8
6
4
2
0
STV_{QT} (ms)
VF-
VF+
*,#
B
Baseline
Occlusion


250
200
150
100
50
0
QT interval (ms)
VF-
VF+
#
#
C
Baseline
Occlusion


350
300
250
200
150
100
50
0
QTcF interval (ms)
VF-
VF+
#
#
D
Baseline
Occlusion


18
16
14
12
10
8
6
4
2
0
EDP (mmHg)
VF-
VF+
#
#
E
Baseline
Occlusion


Baseline
Occlusion
-1000
-2000
-3000
-4000
-5000
-6000
-7000
dP/dtmin (mmHg/s)
#
#
VF-
VF+
F


140
120
100
80
60
40
20
0
ESP (mmHg)
VF-
VF+
#
#
G
Baseline
Occlusion

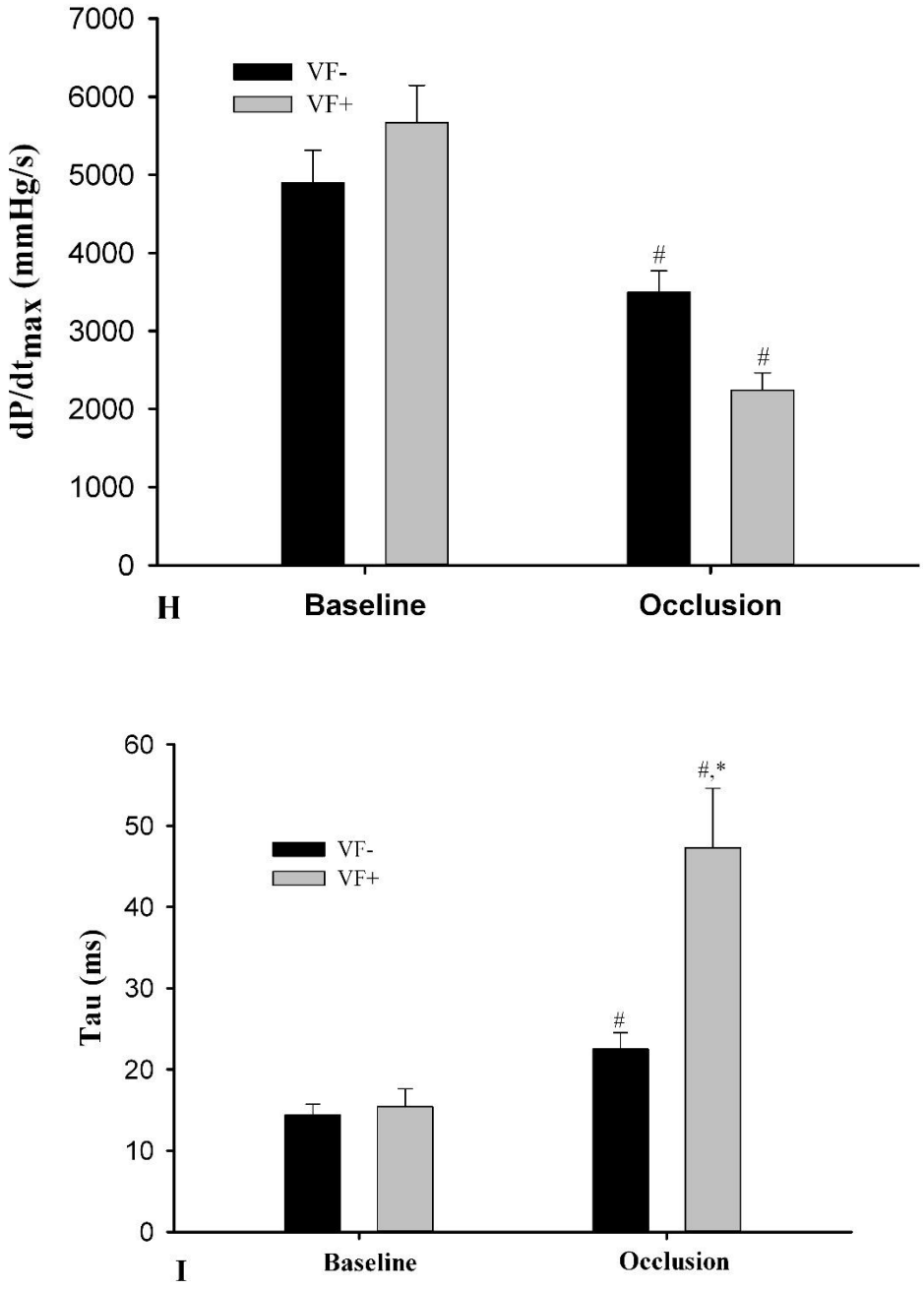


Figure 1. Plots of electromechanical window (EMW), short-term variability of QT interval (STV_{QT}), QT interval, QTcF interval, end-diastolic pressure (EDP), the maximum rate of fall of the left ventricular pressure (dP/dt_{min}), end-systolic pressure (ESP), the maximum rate of rise of the left ventricular pressure (dP/dt_{max}), relaxation time-constant (tau) at baseline and during occlusion. All values are expressed as mean ± standard error of mean. # indicates $p < 0.05$ compared with baseline, * indicates $p <$

0.05 compared between groups (rabbits with ventricular fibrillation, VF+, n=10; rabbits without ventricular fibrillation VF-, n=8), QTcF = corrected QT interval by Fridericia's formula

ROC analysis showed that both QT and QTcF intervals had AUC values less than 0.8 while the ROC analysis of the EMW and STV_{QT} yield AUC values greater than 0.8 (Table 4). Therefore, the QT and QTcF intervals were not valid predictive values for the occurrence of VF. The sensitivities of the EMW and the STV_{QT} were 0.67 and 0.9, respectively. The specificities of the EMW and the STV_{QT} were 0.83 and 0.88, respectively. Therefore, only these two parameters predicted VF with relatively high sensitivity and specificity. The AUC value of the EMW was lower than the value for the STV_{QT}, indicating a relatively lower predictive power. The optimal cut-off values of EMW and STV_{QT} were 64 ms and 5.31 ms, respectively.

Table 4

Predictive power, sensitivity and specificity for QT, QTcF, EMW and STV_{QT}

Parameters	AUC	Sensitivity (%)	Specificity (%)
QT	0.664	60	54
QTcF	0.540	60	58
EMW	0.829	67	83
STV_{QT}	0.963	90	88

AUC; area under the curve; QT; the QT interval; QTcF; the corrected QT interval by Fridericia's formula; EWM; electromechanical window; STV_{QT}; short-term variability of QT interval

In anesthetized rabbits with propofol, the EMW values at baseline were ranging from 23 to 78.8 ms. The current study found that the EMW begins to increase just before the VF develops in VF+ rabbits (Fig. 17). However, the EMW did not change throughout the experiment in VF- rabbits. Similarly, the value of STV_{QT} in the current study was minimal during baseline (ranging from 2.29 to 6.6 ms) and this value was increased just before VF occurred.

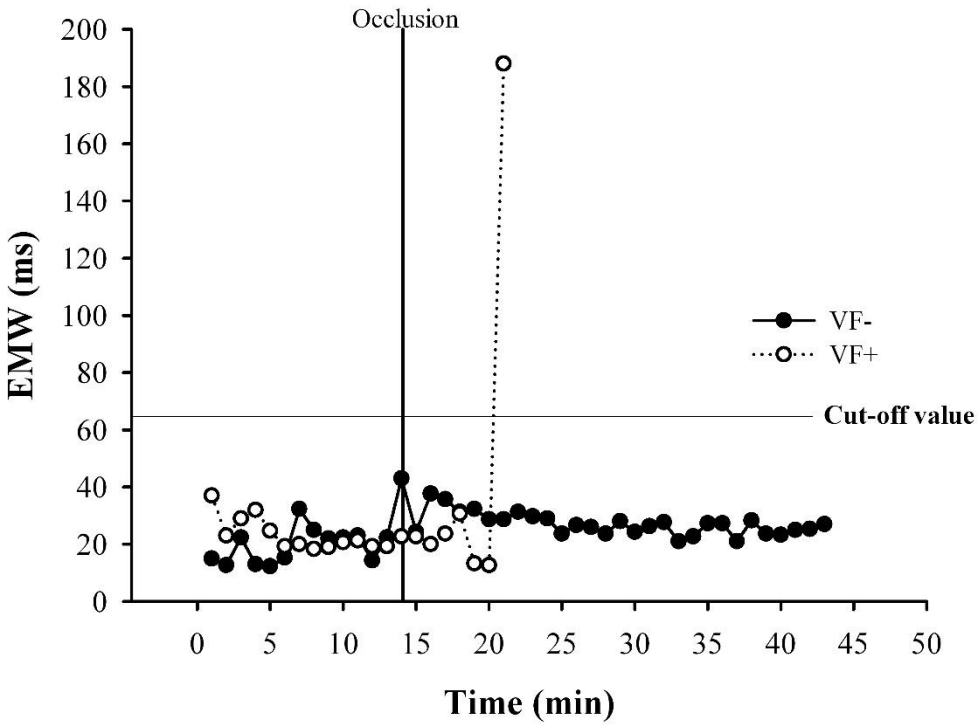


Figure 17. Electro-mechanical window (EMW) on representative of rabbit without VF (VF-) and representative of rabbit with VF (VF+) at baseline and after occlusion by time. Noted that cut-off value of EMW to predict VF is 64 ms.

C. Frequency of arrhythmic beats

The frequency of arrhythmic beats is presented in Fig. 18. At baseline and before coronary occlusion, the arrhythmic beats were zero. During occlusion, the frequencies of R-on-T and VPC in VF+ rabbits had increased significantly when compared with VF- rabbits ($p<0.05$). However, the predictive power, sensitivity and specificity of the arrhythmic frequencies for prediction of VF were lower than 0.8.

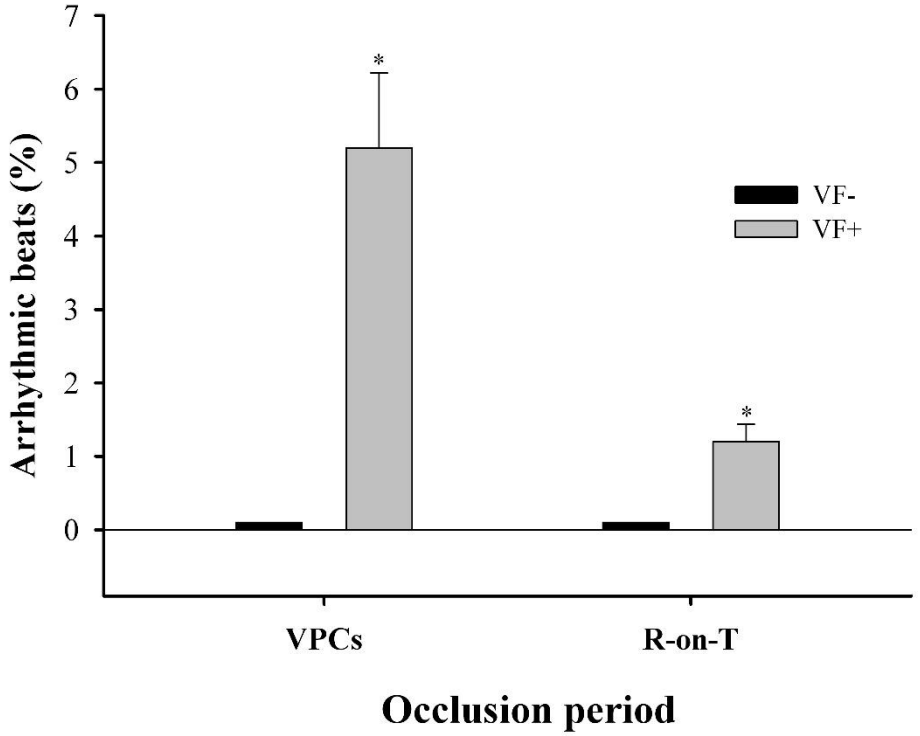


Figure 18. Plot of the frequency of arrhythmic beats including ventricular premature complexes (VPCs) and R-on-T during occlusion period compared between rabbits with (VF+), n=10, and without (VF-) ventricular fibrillation, n=8. All values are shown as mean ± standard error of mean. * indicates $p < 0.05$ compared between groups (VF + versus the VF-)

Study Part 3: Characteristic of EMW in the rabbit model of dofetilide-induced TdP

A. TdP

After administration of dofetilide, 3 out of 6 rabbits showed TdP, or 50% protected by verapamil pretreated (Fig 19). Dofetilide (30 μg/kg/min) was found to have a proarrhythmic potential.

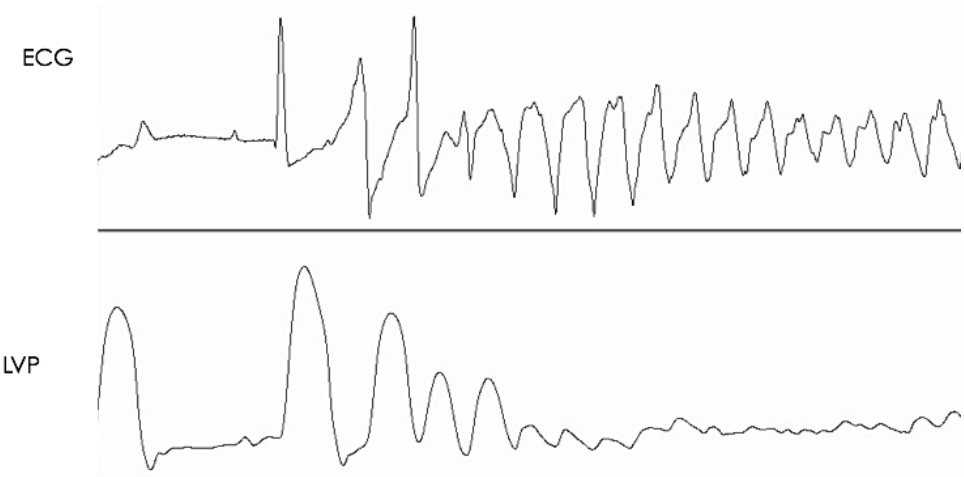


Figure 19. Example of ECG (top panel) and LVP (bottom panel) tracings that demonstrating torsades de pointes (TdP) in the presence of dofetilide (30 μg/kg/min)

B. Effects of verapamil on EMW, QT interval and STV_{QT}

All ECG parameters and LVP parameters reached equilibrium within 10 min. The EMW and other ECG parameters did not change for the verapamil period (Table 5). Verapamil (0.3 mg/kg) led to a significant decrease in dP/dt_{max} and a significant increase in dP/dt_{min} ($p < 0.05$), thereby blocking the L-type calcium channel (Table 6). Figure 20 illustrates the effect of verapamil and dofetilide on EMW in TdP+ and TdP- rabbits.

Table 5

EMW and other ECG parameter after infusion of verapamil and after infusion of dofetilide, n=6

	RR (ms)		HR (bpm)		PR (ms)		QRS (ms)		QT (ms)		QTcF (ms)		EMW (ms)		STV_{QT} (ms)	
	Td P-	Td P+	Td P-	Td P+	Td P-	Td P+	Td P-	Td P+	Td P-	Td P+	Td P-	Td P+	Td P-	Td P+	Td P-	Td P+
Baseline	227±20	225±20	224±16	224±19	64±4	66±5	35±4	45±4	180±6	163±9	278±8	259±9	124±4	228±8	0.7±0.2	0.9±0.2
Verapamil	306±7	268±8	196±4	225±7	78±4	74±5	37±4	46±1	190±5	184±16	282±6	287±28	257±7	188±8	1.5±0.8	0.8±0.2
Dofetilide	348±209	557±41*,#,§	174±99*	115±11*,#	97±4*,#	122±15*,#	55±9	45±9	266±17*,#	277±27*,#	380±19*,#	363±25*,#	-461±17*,#	-612±20*,#	3.2±1	5.1±1

*p < 0.05 as compared to baseline, #p < 0.05 as compared to verapamil, §p < 0.05 as compared to No-TdP rabbits

Table 6

LVP parameters after infusion of verapamil and after infusion of dofetilide, n=6

	EDP (mmHg)		ESP (mmHg)		dPdt+ (mmHg/s)		dPdt- (mmHg/s)		Relaxation time (ms)		Contraction time (ms)	
	TdP-	TdP+	TdP-	TdP+	TdP-	TdP+	TdP-	TdP+	TdP-	TdP+	TdP-	TdP+
Baseline	3±2	2±2	70±4	69±8	3042±115	3215±301	-2804±124	-2803±302	51±2	52±2	42±0.3	42±2
Verapamil	6±3	5±1	61±4	60±2	2071±161*	1796±126*	-2255±189*	-1819±100*,§	54±1	51±1	44±1	45±0.3
Dofetilide	7±4	8±2	69±5	47±1	2542±194	1497±163*,§	-2265±165*	-1391±20*,§	60±0.2*,#	58±2*,#	41±0.6	45±1§

*p < 0.05 as compared to baseline, #p < 0.05 as compared to verapamil, §p < 0.05 as compared to No-TdP rabbits

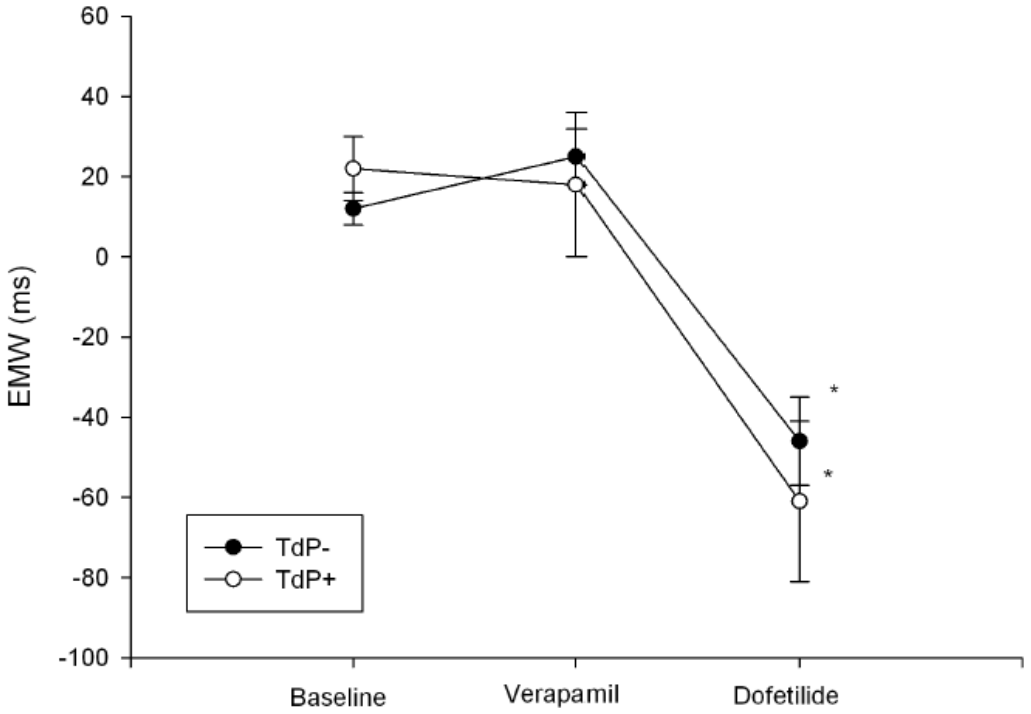


Figure 20. Effects of verapamil and after infusion of dofetilide on mean electro-mechanical window (EMW) in anesthetized rabbits (n=6). * indicates $p < 0.05$ compared with baseline and verapamil. TdP-, rabbits without torsades de pointes (TdP); TdP+, rabbits with TdP.

C. Effects of dofetilide on EMW, QT interval and STV

After dofetilide (30 μg/kg/min) administration, significant decrease in EMW was observed (Table 5, Fig 20) in both groups (TdP+ and TdP-) of rabbits but more negative for TdP+ rabbits. During pretreatment with verapamil and dofetilide infusion, STV_{QT} did not alter either TdP+ or TdP- rabbits (Fig 21).

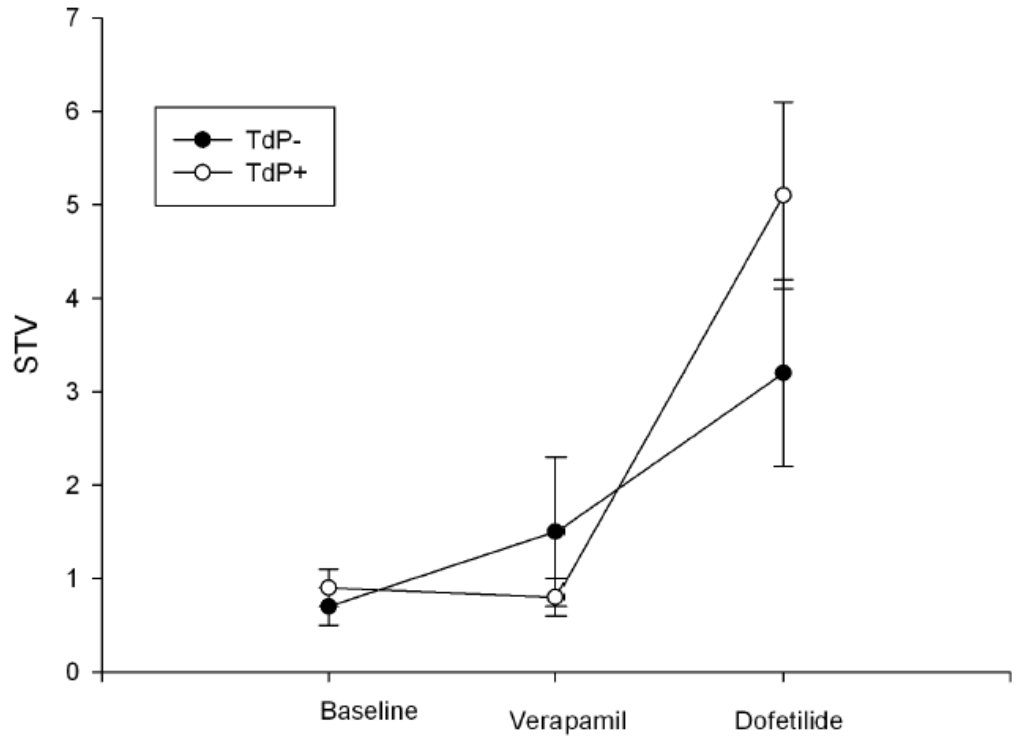


Figure 21. Effects of verapamil and dofetilide on short-term variability of QT interval (STV_{QT}) in anesthetized rabbits. TdP-, rabbits without torsades de pointes (TdP), n=3; TdP+, rabbits with TdP, n=3.

Study Part 4: Effects of preload, contractility, blood pressure, and HR on EMW in anesthetized rabbits

A. Effects of preload on EDP, EMW, QT and STV

In response to incremental of preload as indicated by increased EDP, the EMW did not change from baseline. The EDP was significantly increased from baseline when the normal saline was given at 1.5% of BW. No other finding was observed (Table 7).

Table 7

Electrocardiography (ECG) and left ventricular pressure (LVP) parameters after increasing preload, (n=5)

	Baseline	**0.5%BW**	**1%BW**	**1.5%BW**
RR (ms)	248 ± 15.6	248 ± 15.8	249 ± 13.4	251 ± 15.1
HR (bpm)	245 ± 14.7	245 ± 14.8	243 ± 12.6	270 ± 14
PR (ms)	63.2 ± 5.4	62.8 ± 5.5	59.9 ± 5.6	60.7 ± 6.1
QRS (ms)	51.3 ± 2.4	52 ± 2.1	52.1 ± 2.6	52.5 ± 2.3
EMW (ms)	31.7 ± 6.1	31 ± 6.4	25.1 ± 7.2	30.6 ± 7.1
QT (ms)	153 ± 9.4	154 ± 9.1	158 ± 9.1	158 ± 8.4
QTcF (ms)	244 ± 12.3	245 ± 12.1	252 ± 13.2	250 ± 11.5
EDP (mmHg)	**1.54 ± 0.8**	**0.977 ± 0.8**	**3.05 ± 1.5**	**4.26 ± 1***
ESP (mmHg)	54.5 ± 5.1	55.2 ± 5	58 ± 5.2	59.7 ± 4.2
dPdt+ (mmHg/s)	2704 ± 282	2706 ± 289	2849 ± 203	2883 ± 210
dPdt- (mmHg/s)	-2112 ± 258	-2104 ± 282	-2279 ± 232	-2387 ± 233
STV_{QT} (ms)	2.53 ± 0.7	2.91 ± 1	2.66 ± 0.7	3.17 ± 0.9
Tau (ms)	10 ± 0.6	10.7 ± 0.4	10.1 ± 0.4	9.9 ± 0.3
relT (ms)	51.3 ± 2	51.8 ± 2.3	49.4 ± 0.9	50.1 ± 1.1
ctrT (ms)	42.8 ± 1	42.7 ± 0.5	42.3 ± 0.6	41.6 ± 0.6

*Difference from Baseline, $p<0.05$ using one-way repeated measure ANOVA with Dunnett's test. EMW, electro-mechanical window; EDP, end-diastolic pressure; ESP, end-systolic pressure; dPdt+, dP/dt_{max}; dPdt-, dP/dt_{min}; STV_{QT}, short-term variability of QT interval; Tau, relaxation time constant; relT, relaxation time; ctrT, contraction time.

B. Effects of cilobradine on HR, EMW, QT and STV

Cilobradine significantly decreased HR in a dose dependent manner (baseline 262±10.3 bpm; low dose 189±11.6 bpm; medium dose 148±17.1 bpm; high dose 138±13.6 bpm; $p<0.05$). As a result, the QT interval was lengthened significantly from low (243±11.3 ms), medium (288±14.7 ms) and high (280±13.9 ms) doses when compared with baseline (164±5.1 ms, $p<0.05$). The prolongation of QTcF was also observed in a concentration dependent manner (from 268±6.2 ms to 354±8.56 ms, 386±5.1 ms and 366±12.1 ms, respectively; $p<0.05$). While STV_{QT} was trivially changed in response to cilobradine infusion the EMW was significantly increased at the high dose (baseline 27.54 ms vs 47±9.3 ms) (Table 8).

Table 8

Comparing parameters of electrocardiogram (ECG) and left ventricular pressure (LVP) among the escalating dose of cilobradine (Cilo); 2.5, 5 and 10 mg/kg IV bolus; (n=5).

	Baseline	**Cilo2.5**	**Cilo5**	**Cilo10**
RR (ms)	230 ± 9.2	323 ± 20.7	428 ± 49.3*	453 ± 45.1*
HR (bpm)	**262 ± 10.3**	**189 ± 11.6***	**148 ± 17.1***	**138 ± 13.6***
EMW (ms)	8.58 ± 1.2	-39.1 ± 6.9*	-71.6 ± 14.1*	-71.3 ± 18.7*
QT (ms)	164 ± 5.1	243 ± 11.2*	288 ± 14.7*	280 ± 13.9*
QTcF (ms)	268 ± 6.2	354 ± 8.6*	386 ± 5.1*	366 ± 12.1*
EDP (mmHg)	6.27 ± 1	12.2 ± 3.1	16.6 ± 2.9*	16.8 ± 2.5*
ESP (mmHg)	52.1 ± 0.6	49.9 ± 1.5	50.7 ± 0.8	49.6 ± 1.9
dPdt+ (mmHg/s)	3152 ± 155	2139 ± 271*	1727 ± 84.4*	1693 ± 133*
dPdt- (mmHg/s)	-1615 ± 61	-1532 ± 130	-1411 ± 137	-1408 ± 116
STV_{QT} (ms)	2.73 ± 0.4	2.02 ± 0.4	1.06 ± 0.2	1.64 ± 0.4*
Tau (ms)	16 ± 2.4	17.3 ± 3	20.1 ± 4.4	19.1 ± 3.4
relT (ms)	50.1 ± 0.9	54.1 ± 1.5	57 ± 2.7*	57.6 ± 3.6*
ctrT (ms)	39.4 ± 0.7	40.2 ± 1.3	38.1 ± 1.5	40.1 ± 3.2

*Difference from baseline; $p < 0.05$ using one-way repeated measured ANOVA with Dunnett's test. EMW, electro-mechanical window; EDP, end-diastolic pressure; ESP, end-systolic pressure; dPdt+, dP/dt_{max}; dPdt-, dP/dt_{min}; STV_{QT}, short-term variability of QT interval; Tau, relaxation time constant; relT, relaxation time; ctrT, contraction time.

Pearson regression analysis showed that EMW was significantly correlated with HR with r^2 equal to 0.865 (Fig. 22).

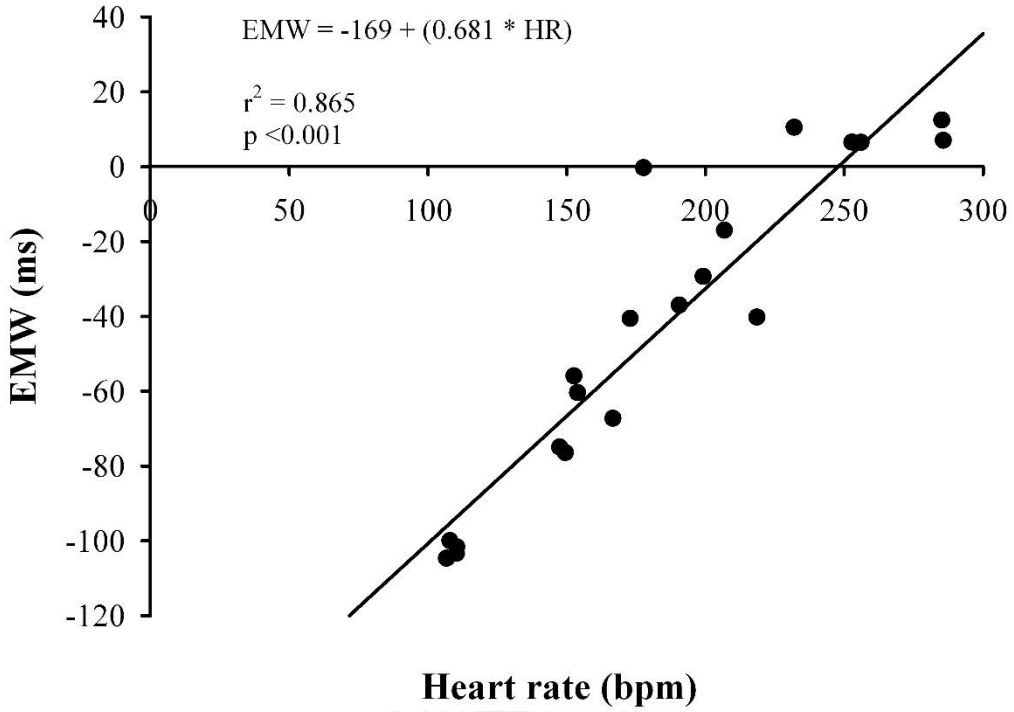


Figure 22. A scatter diagram illustrating the relationship between electromechaical window (EMW) and heart rate (HR). Each point represents heart rate and the corresponding EMW in anesthetized rabbits in each timepoint, n=5. Note that the independent variable is heart rate on the horizontal axis and the dependent variable is EMW on the vertical axis.

C. Effects of phenylephrine and nitroprusside on SBP, EMW, QT and STV

At the highest dose of phenylephrine (mean ESP = 100 mmHg), the EMW of rabbits had increases of >20 ms compared with baseline EMW. The statistically difference also found for the QT interval, EDP, ESP and dP/dt_{min}. No notable findings were observed in other parameters. On the other hand, EMW was not changed when administered with sodium nitroprusside, but the changes in ESP and dP/dt_{min} were observed (Table 9-10).

Table 9

Electrocardiography (ECG) and hemodynamic data under the influence of phenylephrine (PE) to increase blood pressure, n=8.

	Baseline	**PE3**	**PE5**	**PE10**
RR (ms)	243 ± 10.4	251 ± 12.8	260 ± 15.2*	266 ± 15.6*
HR (bpm)	250 ± 10.2	244 ± 11.9	236 ± 13.1*	231 ± 12.9*
PR (ms)	56.6 ± 4	56 ± 2.5	57.1 ± 2.3	59.2 ± 2.8
QRS (ms)	46.9 ± 3.8	46.6 ± 3.8	47.3 ± 3.6	48 ± 2.8
EMW (ms)	27 ± 5.4	27.6 ± 5.6	35.2 ± 9	47 ± 9.3
QT (ms)	155 ± 6	164 ± 9.2	172 ± 10.1*	173 ± 10.1*
QTcF (ms)	248 ± 8.3	260 ± 11	269 ± 11.7	268 ± 12.1
EDP (mmHg)	3.96 ± 1.6	4.87 ± 1.7	7.94 ± 2.3*	9.7 ± 1.5*
ESP (mmHg)	**62.4 ± 4.5**	**74.4 ± 4**	**86.6 ± 7.4***	**100 ± 8.9***
dPdt+ (mmHg/s)	2924 ± 203	3009 ± 193	3111 ± 229	3280 ± 289
dPdt- (mmHg/s)	-2500 ± 252	-2955 ± 203	-3140 ± 190*	-3153 ± 258*
STV_{QT} (ms)	3.52 ± 0.7	3.58 ± 0.9	3.36 ± 0.7	3.44 ± 0.6
Tau (ms)	10 ± 0.6	10.8 ± 0.6	12.3 ± 1.3	15.3 ± 2.1*
relT (ms)	49.9 ± 1.1	52.5 ± 1.7	56.2 ± 3.1*	60.9 ± 3.6*
ctrT (ms)	42.9 ± 0.5	43.9 ± 0.5	44.9 ± 1.6	47.9 ± 1.6*

*Difference from baseline; $p < 0.05$ using one-way repeated measured ANOVA with Dunnett's test. EMW, electro-mechanical window; EDP, end-diastolic pressure; ESP, end-systolic pressure; dPdt+, dP/dt_{max}; dPdt-, dP/dt_{min}; STV_{QT}, short-term variability of QT interval; Tau, relaxation time constant; relT, relaxation time; ctrT, contraction time.

Table 10

Change in blood pressure and other parameters during sodium nitroprusside (SNP) infusions at various escalating doses, n=8

	Baseline	**SNP1**	**SNP3**	**SNP5**
RR (ms)	268 ± 27.2	270 ± 25.6	247 ± 11.2	243 ± 11.5
HR (bpm)	236 ± 17.2	233 ± 16.1	247 ± 10.9	250 ± 11.3
PR (ms)	56.2 ± 2.5	55.9 ± 2.3	52.8 ± 1	51.7 ± 2.1
QRS (ms)	47.2 ± 3.6	47.7 ± 3.6	46.8 ± 3.5	47.3 ± 3.6
EMW (ms)	37.8 ± 10.8	37 ± 10.7	29.2 ± 6.1	21 ± 4.2
QT (ms)	167 ± 7.4	168 ± 7	162 ± 6.2	168 ± 8.4
QTcF (ms)	260 ± 6.8	261 ± 7.4	258 ± 8.2	268 ± 9.7
EDP (mmHg)	6.8 ± 2.5	6.41 ± 1.2	6.1 ± 2.5	6.38 ± 3.1
ESP (mmHg)	75.2 ± 4.9	68.7 ± 4.7	62.5 ± 4.7*	61.1 ± 5.2*
dPdt+ (mmHg/s)	2869 ± 156	2632 ± 146	2602 ± 187	2562 ± 204
dPdt- (mmHg/s)	-2535 ± 228	-2417 ± 263	-2229 ± 222*	-2162 ± 218*
STV (ms)	5.2 ± 1.1	4.02 ± 1.2	5.73 ± 2.6	4.05 ± 1.6
Tau (ms)	13.1 ± 1.3	12.1 ± 1.1	12.6 ± 1.5	12.6 ± 1.9
relT (ms)	56.7 ± 3.3	56.9 ± 3.2	53.5 ± 1.8	53.6 ± 2.5
ctrT (ms)	44.5 ± 0.7	44.9 ± 1	44.1 ± 0.8	44.3 ± 1.1

*Difference from baseline; $p < 0.05$ using one-way repeated measured ANOVA with Dunnett's test. EMW, electro-mechanical window; EDP, end-diastolic pressure; ESP, end-systolic pressure; dPdt+, dP/dt_{max}; dPdt-, dP/dt_{min}; STV_{QT}, short-term variability of QT interval; Tau, relaxation time constant; relT, relaxation time; ctrT, contraction time.

Pearson regression analysis showed that EMW was minimally correlated with blood pressure (Table 11).

Table 11 Linear regression analysis of electromechanical window (EMW), n=17

Model	Coefficient	SE	*t*	Sig	*R*	R^2	Adj. R^2
HR	0.681	0.064	10.7	<0.001	0.93	0.865	0.857
SBP	0.417	0.134	3.12	0.003	0.369	0.136	0.122
dP/dt_{max}	-0.013	0.002	-6	<0.001	0.848	0.72	0.7

HR, heart rate; SBP, systolic blood pressure

D. Effects of Esmolol and Milrinone on dP/dt_{max}, EMW, QT and STV

After administration with esmolol to decrease the myocardial contractility, the ECG parameters (RR, HR, EMW, QTcB and QTcF), and LVP parameters (EDP and dP/dt_{max}) were changed. The EMW was increased when the contractility decreased (Table 12). On the contrary, milrinone administration increased the myocardial contractility, the EMW was not changed. The only parameter that was changed in response to milrinone was $dPdt_{max}$ (Table 13).

Table 12

Effects of esmolol on cardiac contractility and other parameters, n=4

	Baseline	**Esmolol 0.5**
RR (ms)	231 ± 2.2	269 ± 15.2
HR (bpm)	259 ± 2.4	225 ± 12.7
EMW (ms)	12.4 ± 4.9	30.1 ± 0.8*
QT (ms)	167 ± 3.5	169 ± 0.6
QTcF (ms)	273 ± 6	263 ±5.5*
EDP (mmHg)	2.98 ± 0.7	6.41 ± 0.3
ESP (mmHg)	59.8 ± 3.3	59.6 ± 1.8
dPdt+ (mmHg/s)	3247 ± 112	2100 ± 242*
dPdt- (mmHg/s)	-1975 ± 68	-2065 ± 142
STV (ms)	3.14 ± 0.5	3.51 ± 0.6
Tau (ms)	16.7 ± 2.8	16.2 ± 0.2
relT (ms)	57.3 ± 4.2	54.2 ± 0.4
ctrT (ms)	40.1 ± 0.4	44.1 ± 0.1

*Difference from Baseline; $p < 0.05$ with paired t-test. EMW, electro-mechanical window; EDP, end-diastolic pressure; ESP, end-systolic pressure; dPdt+, dP/dt_{max}; dPdt-, dP/dt_{min}; STV_{QT}, short-term variability of QT interval; Tau, relaxation time constant; relT, relaxation time; ctrT, contraction time.

Table 13

ECG and hemodynamic parameters during the milrinone administration, n=4

	Baseline	**Milrinone 1**
RR (ms)	238 ± 7.1	226 ± 5.8
HR (bpm)	252 ± 7.4	266 ± 6.8
EMW (ms)	16 ± 2	11.7 ± 1.9
QT (ms)	170 ± 2.8	168 ± 4.1
QTcF (ms)	274 ± 3.5	275 ± 4.4
EDP (mmHg)	5.98 ± 2.5	6.14 ± 0.3
ESP (mmHg)	60.2 ± 2.5	60.1 ± 2.6
dPdt+ (mmHg/s)	3127 ± 111	3371 ± 50*
dPdt- (mmHg/s)	-1751 ± 145	-1797 ± 173
STV (ms)	2.84 ± 0.4	1.97 ± 0.5*
Tau (ms)	18.9 ± 1.6	18.2 ± 1.7
relT (ms)	56.9 ± 1.1	55.5 ± 0.7
ctrT (ms)	40.8 ± 0.4	41.1 ± 0.3

*Difference from Baseline; $p < 0.05$ with paired t-test. EMW, electro-mechanical window; EDP, end-diastolic pressure; ESP, end-systolic pressure; dPdt+, dP/dt_{max}; dPdt-, dP/dt_{min}; STV_{QT}, short-term variability of QT interval; Tau, relaxation time constant; relT, relaxation time; ctrT, contraction time.

Pearson regression analysis showed that EMW was significantly correlated with dP/dt_{max} with r^2 equal to 0.72 (Fig. 23).

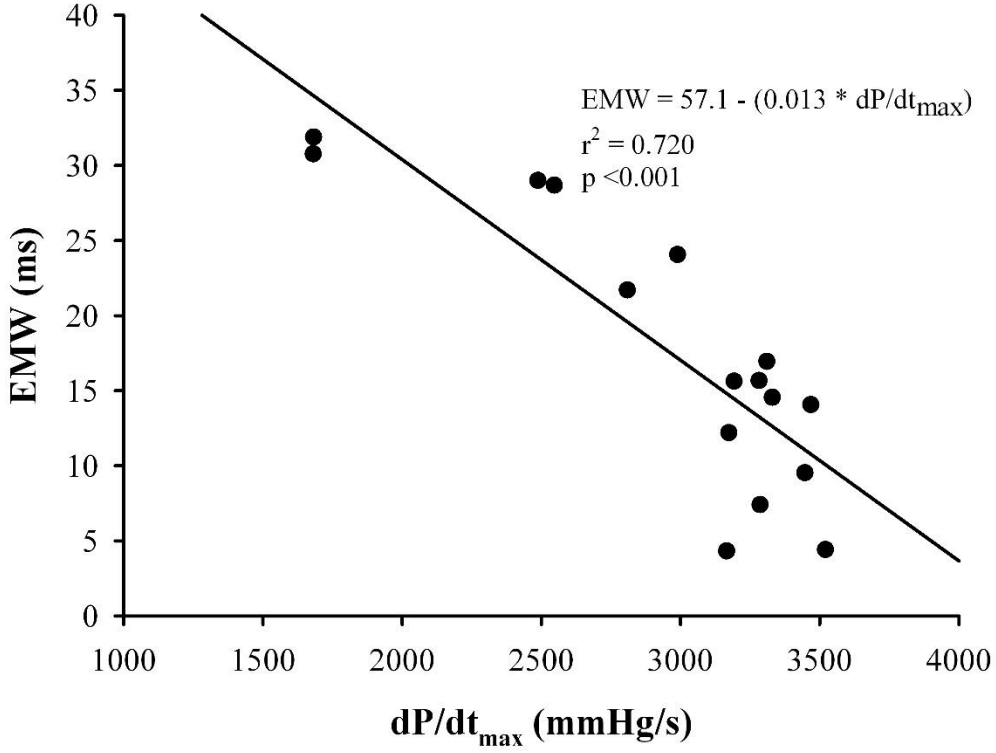


Figure 23. A scatter diagram illustrating the relationship between electromechaical window (EMW) and contractility (dP/dt_{max}). Each point represents dP/dt_{max} and the corresponding EMW in anesthetized rabbits in each timepoint, n=4. Note that the independent variable is dP/dt_{max} on the horizontal axis and the dependent variable is EMW on the vertical axis.

CHAPTER V
DISCUSSION

Study part 1: Characteristic of EMW in animal models of LQT and SQT

The main purpose of this study was to determine the characteristic of EMW in the anesthetized rabbit model of LQT and SQT. 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 was ranging from 1.3 to 53.3 ms. As in previous studies, the diluents used in this study was not affect the studied parameters (van der Linde et al., 2010b). Although, EMW was not difference between TdP-induced rabbit with rabbit without TdP, in response to QT prolonging 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 LQT1. 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., 2010b). 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 different from those values of rabbits that did not develop TdP while receiving QT prolonging drugs. 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; Varro and Baczko, 2011). In LQT1 model, blockades of the I_{Ks} (i.e. HMR1556, JNJ303) were used to trigger TdP (van der Linde et al., 2010b; 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 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 (Kijtawornrat 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 is may be due to the differences in pharmacokinetics and pharmacodynamics of drugs in different animal species (D'Alonzo et al., 1994a; D'Alonzo et al., 1994b; Horinaka et al., 2004). The finding of the current study showed that pinacidil induced VF about 20% of anesthetized rabbit. This is consistent with previous studies in which the use of $I_{K\text{-}ATP}$ openers has been shown to be associated with VF (Chi et al., 1990; Padrini et al., 1992; Lu et al., 2008c). It has been known that $I_{K\text{-}ATP}$ openers shorten action potential duration so that the effective refractory period is abbreviated (Milberg et al., 2007b). Pinacidil has also been shown to increase maximal TDR 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).

The STV_{QT} was used to test for drug induced-TdP (Thomsen et al., 2006). However, STV_{QT} was not change under QT prolonging drugs in our study, while it was increase when receiving the highest dose of pinacidil. One of the weak point of STV_{QT} was the changes of STV_{QT} is only one direction, which is difficult to say that the TdP or VF will occur later. Furthermore, the STV_{QT} has shown a lower relative value from the QT interval, according to previous studies in chronic atrioventricular block dogs (Thomsen et al., 2004; Thomsen et al., 2005).

The use of conscious rabbit model and rabbits with heart diseases to test for torsadogenic compounds has been demonstrated previously (Kijtawornrat et al., 2006a; Kijtawornrat et al., 2006b; Hamlin and Kijtawornrat, 2008; Kijtawornrat et al., 2012). 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. Therefore, the EMW in anesthetized rabbits may be used as a marker for drug-induced QT abbreviation or prolongation.

Limitations of study part 1:

The EMW is an invasive biomarker. Plasma concentrations of drug used were not performed, however, the dosage used was according to previous studies and pilot study. In addition, numerous factors (i.e. heart rate, contractility, blood pressure and changes in preload) may affect the change of the EMW. Therefore, these factors must be evaluated before further investigations on the use of the EMW.

Study Part 2: Use of EMW for predicting ischemia-induced ventricular fibrillation in rabbit with LAD ligation

The main aim of the present study was to investigate whether the changes in the EMW and STV_{QT} can be used to predict the risk of VF in an anesthetized rabbit model of ischemic-induced ventricular arrhythmias. The main findings were that increases of EMW and STV_{QT} were observed just before the VF. These two indices had great predictive power with high sensitivity and specificity for the occurrence of VF. In contrast, changes of the QT and QTcF intervals were not predictive of VF.

Prolonged duration of cardiac action potential duration and consequent lengthening of the QT intervals is antiarrhythmic. However, it has been recognized that not all drugs that lengthen QT and QTc intervals possess antiarrhythmic effects. The lengthening of QT and QTc intervals has been used as a surrogate marker for drug-induced TdP for decades (Hondeghem, 2011). Recent studies suggest that the QTc interval is an unreliable predictor of ventricular arrhythmia (Hondeghem, 2008b; Hondeghem, 2008a). While several novel biomarkers (i.e. iCEB, TRIaD, STV_{QT} and EMW) have been established to predict drug-induced TdP, biomarkers for drug-induced shortening of the QT interval that facilitate re-entry have been overlooked (Shah and Hondeghem, 2005; Thomsen et al., 2006; van der Linde et al., 2010a; Lu et al., 2013). Recently, the absolute beat-to-beat variability and instability parameters of ECG intervals, otherwise known as BVI, have been validated for predicting ischemia-induced VF in an isolated heart preparation (Sarusi et al., 2014). The study demonstrated that QT and QTc intervals did not differ between 'VF+' and 'VF-' groups. However, absolute BVI could be used as a surrogate for VF in preclinical drug investigations.

The present study showed that an increase EMW is associated with VF in the anesthetized rabbit model of ischemia. The EMW quantified the differences between the QT interval and the duration of the mechanical event. To the best of our knowledge, this is the first study designed to evaluate the EMW for predicting VF in anesthetized rabbits. Previous studies have found that a negative EMW is associated with drug-induced TdP in several animal models (van der Linde et al., 2010a; Guns et al., 2012a; Guns et al., 2012b). Those authors also demonstrated that the EMW is not interfered by HR and body temperature. Therefore, the measure has been suggested

for use as a biomarker in preclinical cardiovascular safety studies (van der Linde et al., 2010a).

The present study also demonstrated that an increased STV_{QT} is associated with VF in the model of ischemia. The STV_{QT}, known as beat-to-beat variability of repolarization or BVR, is used to quantify the temporal repolarization liability. Similar results to our findings have been demonstrated in the isolated rat heart, where the elevated STV_{QT} had great predictive power for the occurrence of VF (Sarusi et al., 2014). Previous studies have also found that the increase in STV_{QT} caused by both cardiovascular and non-cardiovascular drugs can be used to predict drug-induced TdP (Thomsen et al., 2004; Thomsen et al., 2006; Gallacher et al., 2007). Even as the detailed mechanisms of drug-induced TdP and VF are different, they share a broader common mechanism. Functional re-entry could explain the elevated STV_{QT} in both types of arrhythmias (Gaztanaga et al., 2012).

An increased frequency of arrhythmic beats is associated with increase heterogeneity of repolarization, and has been suggested to be a substrate for the re-entry pathway, and a trigger for arrhythmias (Choi et al., 2002; Boulaksil et al., 2011). Recently, the frequency of arrhythmic beats and R-on-T were found to be good predictors of the occurrence of VF in isolated rat hearts (Sarusi et al., 2014). However, the complexity of arrhythmic beats was suggested to be more important for the mechanism of drug-induced TdP than the frequency of arrhythmic beats in an α_1-adrenoceptor-stimulated, anesthetized rabbit model (Farkas et al., 2010). In the present study, the frequency of arrhythmic beats (i.e. R-on-T and VPC) in the VF+ rabbits were noticeably greater during coronary occlusion when compared with baseline or VF- rabbits.

Anesthetized rabbits were used because there is relatively similar profile between the potassium currents in rabbits and those in humans, although the HR is differs (Dumaine and Cordeiro, 2007). In the current study, coronary occlusion produced reductions in HR, ESP and dP/dt_{max} whereas the QT, QTcF, EDP, dP/dt_{min} and tau were increased. These results indicate that the model of acute myocardial ischemia performed by coronaries ligation was successfully established. Similar results

were found in previous works of rabbits and other species (Corr et al., 1976; Scherlag et al., 1982; Brown et al., 2014; Wang et al., 2017).

Limitations of study part 2:

The EMW is an invasive biomarker. Rabbit models of ischemia are one of many other models of ventricular arrhythmias. Therefore, further studies should be performed in several animal models to confirm our findings. In addition, numerous factors (i.e. heart rate, contractility, blood pressure and changes in preload) may affect the change of the EMW. Therefore, these factors must be evaluated before further investigations on the use of the EMW in humans.

In conclusion, this study shows that, in comparison with the measure of QT/QTc intervals, changes in the EMW and STV_{QT} are more sensitive to the development of VF in rabbit models, but these do not change in rabbits without VF. The present study is an early step in the validation of the EMW as a predictive measure, and illustrates the feasibility of using the EMW and STV_{QT} in predicting the risk of VF created by the ligation of coronary arteries.

Study Part 3: Characteristic of EMW in the rabbit model of dofetilide-induced TdP

Current regulatory guidelines for evaluating the potential of new chemical entities that cause long QT-related arrhythmogenicity focus on the relationship among I_{Kr} block, QT prolongation and afterdepolarization (Fermini and Fossa, 2003; Fenichel et al., 2004). The data demonstrated that the EMW under the verapamil, the L-type Ca^{2+} channel blocker, dofetilide, the pure I_{Kr} blocker, administration did not different between groups; however, the EMW tended to be more negative in rabbit presented with TdP. These findings have important implication for evaluation of the arrhythmiogenic risk marker and may contribute to use of EMW in early drug development.

Dofetilide is one of a family of methanesulfonanilides that include sotalol, E-4031 and almokalant that are potent I_{Kr} blockers. These drugs have a relatively high propensity to cause TdP. As previously shown, verapamil was effective to prevent TdP, whether it stems from congenital or acquired LQT (Shimizu et al., 1995; Aiba et al., 2005; Gallacher et al., 2007). The common Ca^{2+} antagonism of verapamil was used to investigate whether they could improve repolarization reserve, as reflected in protection against dofetilide-induced TdP. In the current study, verapamil prevents 50% (3 out of 6 rabbits) of rabbits from dofetilide triggered-TdP. This result was in contrary to other studies. The animals pretreated with verapamil had a highly protective effect on TdP induction, most likely related to inhibition of I_{CaL} (Gallacher et al., 2007; Oros et al., 2010). The I_{CaL} has been studied extensively because a blockade of I_{CaL} by verapamil prevented development of EADs, which considered as a possible initial mechanism for TdP in LQT syndrome (January et al., 1988; Belardinelli et al., 2003; Sims et al., 2008). This effect may also explain why I_{Kr} blockade by verapamil is not proarrhythmia because an additional blockade of I_{CaL} protects the heart from developing EADs (Zhang et al., 1999). The balance between I_{CaL} recovery and ventricular repolarization serves a physiological stabilizer (Guo et al., 2008). Moreover, EADs and calcium transients have been related (Hamlin and Kijtawornrat, 2008). Clinical data have also shown that verapamil prevents EADs and ventricular arrhythmias induced by epinephrine in congenital LQT syndrome patients (Shimizu et al., 1995).

The preventive effect of verapamil in our study supports the thought that Ca^{2+} influx via I_{CaL} channel plays a pivotal role in induction of TdP. This protective effect may be a direct effect of verapamil on I_{CaL} channels or its effects on reduction of ventricular transmural dispersion of repolarizatioin (Milberg et al., 2005). Pretreatment with verapamil was also associated with extension in relaxation time, and reductions in dP/dt_{max}, dP/dt_{min} and the torsadogenicity of dofetilide. This indicates that verapamil may influence the element of EMW (i.e., lengthening of $QLVP_{end}$ in LVP tracing). The QT interval in this present study was not change during verapamil dosed, but largely increased during the dofetilide administration. However, the effectiveness of an intervention to abbreviate the QT interval is not necessarily harmonic with its efficacy to reduce the incidence of arrhythmogenesis (Milberg et al., 2005).

The present study illustrates that highly negative EMW are shown in dofetilide-treated anesthetized rabbits, especially in TdP+ group. It may cause form the study protocol; however, when specifically observed at the verapamil-treated time point. No change of EMW was observed. It was according to the QT interval changed during the experiment. These results together with earlier reports showed that QT prolongation did not correlate with proarrhythmic liability of repolarization-prolonging drugs (Eckardt et al., 2002; Milberg et al., 2002). Other ECG parameters, such as PR and QRS intervals, were tend to increase while verapamil was administered, which indicates the effect of Ca^{2+} channel blocker. Then, it appears that the changes of the EMW are driven by the changes of the QT interval as in study part 1.

The STV_{QT} has been shown to predict the occurrence of drug-induced TdP in anesthetized rabbits (Lengyel et al., 2008). In contrast, the STV_{QT} failed to predict drug-induced TdP with adrenergic stimulated anesthetized rabbits (Vincze et al., 2008). Thus, the predictive power of an increase in the STV_{QT} is debatable. In our study, dofetilide administration increased STV_{QT} both in TdP+ and TdP-. On the other hand, the STV_{QT} did not differentiate the occurrence of TdP. Thus, the predictive value of an increase in STV in anesthetized rabbits is questionable.

Limitations of study part 3:

The number of animals used in the study is limited; therefore, more animals are needed to ensure the findings.

Study Part 4: Effects of preload, contractility, blood pressure, and heart rate on EMW in anesthetized rabbits

This study investigated the impact of various factors (i.e. preload, contractility, blood pressure and heart rate), on the EMW in anesthetizes rabbits. The primary endpoint was observed when EMW started to change. This study demonstrated lack of an effect of preload and blood pressure on the EMW. On the other hand, other factors affected the EMW in various level.

Normal saline administered at the 0.5, 1, and 1.5% of BW was not associated with EWW changing in anesthetized rabbits. Analysis of normal saline load-response relationship indicated that preload is unlikely to cause EMW differentiation. These results show that preload has no effect on the EMW at an IV dose of 0.5, 1, and 1.5% BW, which is greater than the normal volume administered on drug testing.

The EMW values differed for blood pressure changed. These results can be explained by the fact the hemodynamic effects showed considerable spontaneous variations. The mean BP of phenylephrine administered was significantly higher than baseline, 62.4 ± 4.5 mmHg and 100 ± 8.9 mmHg, respectively. Mean BPs of baseline and sodium nitroprusside administered were 75.2 ± 4.9 mmHg and 61.1 ± 5.2 mmHg ($p < 0.05$), respectively. In phenylephrine study, the EMW was significantly increased in according to increasing of blood pressure, from 27 ± 5.4 ms to 47 ± 9.3 ms. The QT interval, end diastolic pressure and dP/dt_{min} were increased. Other parameters showed no significant difference between baseline and escalating doses of phenylephrine. In the sodium nitroprusside study, the EMW was not significantly changed, unless it tend to decrease, but it had a largely variation. Only dP/dt_{min} was changed during the drug administration. Other parameters showed no significant difference between baseline and escalating doses of sodium nitroprusside.

In this study, phenylephrine and sodium nitroprusside were administered by IV infusion. The rapid increase of the phenylephrine concentration caused hypertension. Phenylephrine, an α_1-adrenoceptor agonist, significantly increased QT interval in human hearts (Magnano et al., 2005). Electrophysiologically, phenylephrine induced a reduction of the I_{to} in ventricular myocytes from various species (Apkon and Nerbonne, 1988; Braun et al., 1990). Previous studies in rabbit hearts showed that the I_{Kr}, not I_{to},

was considered to be the major ionic current that is responsible for the QT prolongation (Zhang et al., 2007). In this study, the EMW, which was calculated from the QT interval and $QLVP_{end}$, was increased. It showed that EMW changed under influence of blood pressure was not change from the QT interval.

Sodium nitroprusside reduced left ventricular filling pressure, systemic vascular resistance blood pressure fell, and increased cardiac output in dogs (Prewitt et al., 1981; Prewitt and Wood, 1981). BP decreased rapidly after starting sodium nitroprusside infusion because of its potential effect, and then BP slowly decreased even though the infusion continued, it may cause of homeostatic feedback mechanism. BP is controlled by a balance of the sympathetic and parasympathetic nervous system through the actions of the vasomotor center and baroreceptors in the body.

EMW after phenylephrine administration was increased 1.74-fold from baseline, and sodium nitroprusside was not caused a significantly changed. The outlines of these effects were observed after phenylephrine administration in a similar manner, increased BP, increased EMW. EMW was prolonged to 47 ms. Therefore, EMW should be used with cautious to account for its blood pressure effects.

Esmolol is a short acting cardio-selective β_1-blocker expected to have negative chronotropic effect. Intravenous administration of esmolol to telemetered non-human primates was associated with significant decreases in heart rate and arterial pressure (Authier et al., 2007). In our study, the anesthetized rabbits showed a significant decrease in heart rate but no effect on end systolic pressure. The lengthening of the EMW was observed in esmolol-treated rabbits when compared with baseline, while the EMW was not changed when the rabbits received milrinone. Other parameters except dP/dt_{max} were not changed under milrinone administration. On the other hand, the QT interval was not changed in esmolol study. This is, in general, the EMW changed in this case was come from QLP_{end} interval, although the LVP parameters did not significantly change. After esmolol administration, the contraction of the ventricle was significantly decreased. We did not found the changed in relaxation of the ventricle. This study showed that only decreasing in contractility affects the EMW values.

However, from this study we are unable to identify the precise mechanism for the increased in EMW.

Recently, the EMW was shown that it is not altered by heart rate changes or body temperature fluctuations, which can influence the QT interval (Guns et al., 2012b). In the current study; however, it appears that the changes of the EMW are affected by decreased heart rate from cilobradine administration. Cilobradine is an I_f channel blocker. It was found that when the sinus rate is lowered, the diastole duration is prolonged (Kedem et al., 1990). Recently, the QTc prolongation has been previously observed in rabbits with cilobradine administration (Panyasing et al., 2010). In present study, the QTc interval was prolonged, and the EMW was decreased to negative value as in the LQT study. It can imply that the changes in EMW may affect from changes in heart rate, which is a result from QT interval. Furthermore, the I_f channel blocker was found that it had an inotropic effects in the mammalian heart, it can exert either positive or negative inotropic effects (Boldt et al., 2010). Moreover, in isolated spontaneously beating rabbit hearts, cilobradine reduced contractility as seen in our study (Granetzny et al., 1998). In contrast, a positive inotropic effect in left atrial preparations was noted (Boldt et al., 2010). It is well known that a reduction of the beating rate can reduce force of contraction (Koch-Weser and Blinks, 1963). This is the classical staircase or treppe phenomenon. Negative chronotropic effects have been reported using telemetry in the mouse for ivabradine and cilobradine (Du et al., 2004; Stieber et al., 2006). The effect is probably not due to an effect on the I_f current: the IC_{50} value for the inhibition of the I_f current is around 2 μmol/l (Bois et al., 1996). At higher concentrations, it can block L-type Ca^{2+} channels. Stimulation of L-type Ca^{2+} channels exerts positive inotropic effects, but inhibition leads to a negative inotropic effect. Then the changes of EMW in this study may affect from the effects of both heart rate and contractility, as the side effects of physiological responses to drugs used in this study.

Limitations of study part 4:

Plasma concentrations of drugs were not measured; however, drugs were given intravenously and doses are recommended in the literatures and those have used previously in our pilot studies. Moreover, the results in anesthetized rabbit may differ from other species, as the different in cell electrophysiology in dogs and guinea pigs.

CHAPTER VI

SUMMARY

The EMW is a biomarker that has been proposed for predicting the risk of drug-induced TdP. TdP is a polymorphic ventricular tachycardia that may lead to reduction of cardiac output, development of VF and sudden cardiac death. VF is the most serious cardiac arrhythmia resulted in sudden death if defibrillation does not apply. The overall objective of the present study was to investigate potential for EMW to use as a surrogate marker for TdP and VF in LQT, SQT, and ischemic condition. Firstly, the characteristics of EMW in animal models of LQT and SQT were determined. Rabbits were randomly given the reference compounds either compounds known to lengthen human QT intervals (i.e. dofetilide, sotalol and quinidine), compounds known to shorten human QT intervals (i.e. nicorandil, pinacidil and cromakalim) or vehicle-treated control. The results showed that all parameters of ECG and LVP in vehicle-treated rabbits were unaltered. All three drugs known to lengthen QT intervals prolonged QT and QTcF intervals while the EMW were markedly decreased to negative values. Pinacidil shortened QT and QTcF intervals significantly while the EMW was increased to more positive values. This study also demonstrated that the EMW is associated with QT intervals suggested from a high r^2 of Pearson correlation. It can be concluded that the EMW is negative in the present of QT prolonging drugs while it is more positive in the present of QT shortening drugs. Interestingly, not all QT prolonging drugs induced TdP. Only dofetilide produced marked negative EMW and induced TdP. Similarly, not all QT shortening drugs induced VF. Only pinacidil produced sharpedly increased EMW and induced VF. Therefore, EMW can be used as a biomarker for drug safety evaluation as its changes reflect drug-induced QT prolonging and drug-induced QT shortening in addition to QT or QT intervals. Moreover, the results suggest that an anesthetized rabbit can be used in Safety Pharmacology studies to assess liability of QT intervals and EMW.

Secondly, the study was designed to determine the use of EMW for predicting ischemia-induced VF in anesthetized rabbits produced by coronaries ligation. In rabbits with VF, the EMW was significantly higher than in rabbits without VF. STV_{QT} had

significantly increased before the onset of VF in rabbits that experienced VF, but not in rabbits that did not experience VF. The EMW and STV_{QT} had better predictive powers for VF with higher sensitivity and specificity than the QT and QTcF measure. From ROC analysis, the result suggested that the increasing of EMW as well as the elevation of STV_{QT} could potentially be used as biomarkers for predicting of VF in anesthetized rabbits with myocardial ischemia.

Next, the study was designed to determine the characteristic of EMW in the rabbit model of dofetilide-induced TdP. The results showed that verapamil can suppressed TdP. Verapamil significantly reduced dP/dt_{max} in both groups of rabbits (TdP+ vs TdP-); however, the level of decrease did not affect the EMW. Dofetilide decreased EMW to negative values both in rabbits with and without TdP development in which it decreased more for TdP+ groups. Since the number of animal per group was a limitation of this study, more animals are needed to ensure our findings. Further study involving alteration in calcium homeostasis of myocytes should be performed.

Finally, the last study was designed to evaluate several factors (i.e. preload, HR, BP and contractility) that may affect the changes of EMW value. The rabbits were randomly divided to receive one of the treatments: 1) preload (escalating volume of normal saline), 2) HR reduction by escalating intravenous infusion with cilobradine, 3) changing BP by intravenous infusion with phenylephrine and sodium nitroprusside and 4) changing contractility by intravenous infusion with esmolol and milrinone. All ECG and LVP parameters were recorded. The results showed that intravenous infusion with normal saline significantly increased preload as indicated by elevated EDP but it did not affect the EMW. Intravenous infusion with cilobradine caused significantly decreased in HR while the QT and QTcF intervals were significantly lengthened. As a result, the EMW was significantly reduced (become negative) in a dose dependent manner. The Pearson correlation analysis demonstrated a high correlation between EMW and HR ($p<0.001$, $r^2 = 0.865$). PE significantly increased systolic blood pressure in a dose dependent manner. At the highest dose of phenylephrine, the HR was significantly decreased from activation of baroreceptor reflex; however, the EMW was trivially changed. SNP infusion caused significant reduction in SBP without any change in HR and other parameters. The Pearson correlation showed no relationship between

changes of BP and EMW. Intravenous infusion of esmolol produced a significant reduction in dP/dt_{min} without any effect on HR or blood pressure. On the other hand, intravenous milrinone administration caused a significant increase in dP/dt_{max}. Pearson correlation showed a closed relationship between contractility index (dP/dt_{max}) and EMW. These results indicated that, in the anesthetized rabbit model, EMW was affected by changing of HR and contractility but not BP. Therefore, interpretation of changes of EMW in this model should be cautious. The HR and contractility index should be measure together with EMW when using EMW as a biomarker for predicting arrhythmogenic risk in anesthetized rabbits.

Benefit of the study

As far as the authors know, the current study is the first to evaluate the use of EMW in anesthetized rabbit model. The crucial and novel finding of these studies are that the EMW can be used as a surrogate marker for predicting the TdP and VF in LQT, SQT anesthetized rabbit models and in ischemia-induced VF rabbit model. However, the EMW was affected by extremetly changes of HR and contractility. Therefore, care should be taken when use EMW especially when the animals were out of normal physiological conditions. Interestingly, the EMW is superior than QT/QTc intervals when tested in ischemia-induced VF rabbit model.

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