**INTRODUCTION**

The QT interval is used for drug development and by clinicians as a surrogate marker for the prediction of a serious adverse drug effect, torsades de pointes (TdP) (Roden, 2004). Lengthening of QTc is believed to be both antiarrhythmic and proarrhythmic changes in class IA and class III antiarrhythmic drugs (Hondeghem et al., 2001). The relationship between the drug-induced prolongation of QTc interval and risk of proarrhythmia is not fully elucidated (Valentin et al., 2004). Recent studies suggested that prolongation of QTc interval without the presence of TRIaD (Triangulation of the action potential, Reverse use dependence, Instability, and Dispersion of action potential) is antiarrhythmic (Hondeghem et al., 2006; Milberg et al., 2007). Lengthening of QTc is the common signal to indicate the potential of a therapeutic agent to produce TdP. Indices of proarrhythmic activities (i.e., electrical instability and dispersion of repolarization, reverse use-dependency, and change in the shape of the action potential) have been suggested to be important predictors of arrhythmogenicity (Valentin et al., 2004). However, as with many surrogate markers, the relationship to the event of interest is still imperfect and needs further clarification.

It is well-known that patients with heart failure are predisposed to TdP. Recent evidences suggested that calcium sparks, in myocytes isolated from failing rabbit hearts produced by coronary ligation, are reduced and asynchronous resulting in slowed kinetics of calcium transients (Hamlin and Kijtawornrat, 2008; Kijtawornrat et al., 2010). Abnormalities in intracellular calcium handling (i.e., spontaneous release of calcium from the sarcoplasmic reticulum) play major roles in the genesis of triggered activity (Janse, 2004). Furthermore, reentrant excitation within the network of surviving myocardial...
fibers in the infarct has been revealed in the failing heart with a healed infarct, and has been thought to be a key factor producing ventricular arrhythmias. Both triggered activity and re-entry are assumed to be operative in the pathogenesis of TdP (Noda et al., 2004).

It has been shown that rabbit with failing heart not merely lengthens the QT and QTc intervals in response to a potential therapeutic agent but also develops TdP (Kijtawornrat et al., 2006; Panyasing et al., 2010). However, the utilization of isolated failing rabbit hearts for drug safety assessment has not been evaluated. Thus this feasibility study was conducted to determine if known QT-lengthening drugs (cisapride, dofetilide, and quinidine) might actually produce QT interval prolongation in an isolated failing rabbit heart perfused with the modified Krebs-Henseleit solution.

### MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Faculty of Veterinary Science, Chulalongkorn University and conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institute of Health.

#### Heart failure induction

Twenty five male New Zealand White rabbits were anesthetized with ketamine (35 mg/kg) and xylazine (5 mg/kg) administered intramuscularly. Animals received 100% oxygen (at a rate of 400-600 ml/min) and 0.5 to 1.0% isoflurane through a loose-fitting face mask designed for a small animal. The details of surgery were previously described (Kijtawornrat et al., 2006). Briefly, through a median sternotomy and pericardiotomy, the left anterior descending and the descending branch of the left circumflex coronary arteries were ligated. The pericardium remained opened but the thorax was closed. Four rabbits developed fatal ventricular arrhythmias during the procedure. The remaining 21 animals received ketoprofen for analgesia and enrofloxacin for prophylaxis against infection for 3 days. They made uneventful recoveries. Sham-operated control animals (Twenty one male New Zealand White rabbits) were treated and handle identically, with the sole omission of placing the suture around the coronary arteries. The duration for the development of myocardial failing (4 weeks) was selected based on previous publication of Litwin and colleagues (2000). The authors demonstrated that rabbits with coronary ligation for 4 weeks showed abnormal kinetics of contractions and altered Ca^{2+} transients when compared to normal rabbit.

Both before surgery and at 4 weeks after surgery echocardiographic examination was performed under light ketamine/xylazine sedation (15 mg/kg and 3 mg/kg, respectively). The rabbit was placed in right lateral recumbency with an area denuded to allow images to be obtained from the dependent right hemithorax. Imaging was performed using an Aloka SSD-1400 Echocardiographic System (Aloka America, Wallingford, CT, USA) with a 5 MHz transducer. Echocardiograms were obtained with simultaneous electrocardiograms (ECG), and all raw data were captured digitally for off-line measurement. Left ventricular function was assessed by measurements of left ventricle wall thickness and internal dimensions during systole and diastole, which allowed for calculation of shortening fraction (SF). A shortening fraction reduction of 15% from baseline was used as an entry criterion to document the failing heart.

#### Isolated heart preparation and ECG recording

The procedures, which have been described before (Hamlin et al., 2004; Kijtawornrat et al., 2005), were undertaken in accordance with USDA regulations. Hearts were removed from 42 male rabbits (heart failure group, n = 21; normal group, n = 21; weighing 2.0-2.5 kg). They were anesthetized with ketamine (35 mg/kg) and xylazine (5 mg/kg) and anticoagulated with sodium heparin (100 U) injected intravenously. Animals were intubated and ventilated with room air using intermittent positive pressure. Hearts were exposed through a median sternotomy. The hearts were removed in less than a minute and suspended on the Langendorff perfusion apparatus. The modified Krebs-Henseleit solution, gassed with 95% oxygen and 5% carbon dioxide, with pH of approximately 7.4, temperature of approximately 37 ± 1.0°C, and a perfusion pressure of 84 mmHg, was used as perfusate. The modified Krebs-Henseleit solution was composed of the following (mM): 118 NaCl, 4.6 KCl, 11.0 glucose, 24.9 NaHCO₃, 1.1 MgSO₄, 2.5 CaCl₂, and 1.1 KH₂PO₄. This solution mimics, closely, the electrolyte concentration in the normal rabbit and has been widely used for isolated rabbit heart by several investigators (Hondeghem et al., 2001, 2003; Milberg et al., 2007). All hearts were perfused according to the methods of Langendorff and were instrumented to measure the left ventricular isovolumetric pressure with a fluid-filled balloon connected to a precision pressure transducer inserted through the mitral annulus. A bipolar transcardiac electrogram was obtained from electrodes placed on the right atrium and the apex of the left ventricle. This electrode configuration produces a low noise, high gain signal from which QT interval can be measured accurately, and the bipolar transcardiac configuration possess the advantage over unipolar recordings of...
representing the entire heart rather than a more localized epicardial volume. Recordings were made on an EMKA IOX data acquisition system version 2.4.2.6 (EMKA technologies, Falls church, VA, USA) with a frequency response between 0.01-500 Hz (sampling rate of 1 kHz).

**Drugs**

Cisapride, dofetilide, and quinidine were purchased from Sigma-Aldrich. Cisapride was dissolved in 1 ml of acetic acid and diluted up to 1 l of the modified Krebs-Henseleit solution to produce a 10 μM stock solution. Dofetilide was dissolved in the modified Krebs-Henseleit solution with a small drop (~10 μl) of 0.5 N HCl to produce a 10 μM stock solution. Quinidine was dissolved in the modified Krebs-Henseleit solution to produce a 10 μM stock solution. Molar concentrations of cisapride, dofetilide, and quinidine were collected by diluting from the stock solution. Acetic acid in the modified Krebs-Henseleit solution was prepared so that it could be infused into the vehicle at a concentration identical with those for each molar concentration of cisapride.

**Experimental protocol**

All isolated hearts were allowed to equilibrate for 15 min before baseline measurements were taken. To evaluate the effect of time on isolated rabbit hearts, five normal hearts and five failing hearts were perfused continuously for 60 min with vehicle (the modified Krebs-Henseleit solution plus acetic acid). Thirty two hearts (16 failing hearts and 16 normal hearts) were perfused for 60 min with 15 min at each of the following escalating concentrations of cisapride (n = 5), dofetilide (n = 6), and quinidine (n = 5): 0, 0.01, 0.1, 1, and 10 μM. Physiological measurements were made during the final 1 min of each 15 min infusion of each concentration. Measurements for all physiological parameters (RR, QT, dP/dtmax and dP/dtmin) were made using the ECG auto 2.1.0.8 (EMKA technologies, Falls church, VA, USA). These parameters represent the important electrophysiological and mechanical properties of the heart, and they monitor accurately ventricular repolarization and documented a failing heart when present. The QT interval was measured from the beginning of QRS complex until the end of T wave. QT was corrected for RR interval by dividing each QT by the cube root of the preceding RR interval according to the Fridericia correction, QTc(F) (Fridericia, 1920). This method was used to correct QT for RR interval because it has been shown in dogs and humans to produce a QTc relatively independent of RR interval over RR intervals varying well beyond those obtained in this study (Hamlin et al., 2004).

**Statistics**

Means and SEM’s of each parameter were calculated. Means were compared by two ways ANOVA with repeated measures design on dose or on time (for vehicles). When a significant F-statistic was established, specific means were compared using a Tukey post-hoc test requiring a P < 0.05 for significance. Plots of mean values (± S.E.M.) for each group of animals were made for all parameters versus concentrations and time.

**RESULTS**

After 4 weeks, all remaining rabbits with coronary arteries occlusion (n = 21) developed myocardial infarction notice from myocardial dyskinesia during echocardiogram assessment and went into a stage of failing heart with a reduced shortening fraction 17.7% from baseline (post-surgery 24.32 ± 0.51% vs pre-surgery 29.54 ± 0.43%). Shortening fraction of rabbits with sham operation did not change from baseline (post-surgery 30.25 ± 0.33% vs pre-surgery 29.14 ± 0.82%).

In the Langendorff preparation, all hearts survived exposures through a 10 μM concentration, and no heart developed ventricular arrhythmia including torsades de pointes. Conduction disturbance (i.e., 1st, 2nd, and 3rd degree AV block) developed when hearts were exposed to all three QT-prolonging drugs at 10 μM.

The RR intervals were not different at baseline between normal and failing hearts (369 ± 25 vs 363 ± 39 ms, respectively). In response to vehicle perfusion, vehicle given over time had no effect on RR interval for each group; however, RR interval of normal hearts was significantly longer than failing hearts at 45 and 60 min (p < 0.05). At the highest concentration, cisapride and quinidine lengthened RR interval of normal hearts more than failing hearts. In response to escalating doses of all three torsadogens, RR interval increased significantly (p < 0.05) in a dose-dependent manner (Fig. 1). Neither QT nor QTc(F) were significantly different during baseline between normal and failing hearts (189 ± 11 and 264 ± 11 ms vs 186 ± 21 and 259 ± 21 ms, respectively). Vehicle perfusion over time had no effect on QT or QTc(F) in either normal and failing hearts (Figs. 2 and 3). Cisapride at 10 μM significantly lengthened QT and QTc(F) in normal hearts more than failing hearts. All three QT-prolonging drugs significantly prolonged QT and QTc(F) in a concentration dependent manner for both normal and failing hearts (p < 0.05).

During baseline, before exposure to any test articles, dP/dtmax was significant lower (p < 0.05), and dP/dtmin was significant higher (p < 0.05) in failing hearts than in nor-
DISCUSSION

The purpose of this study was to determine if known QT-lengthening compounds (cisapride, dofetilide, and quinidine) might actually prolong QT and QTc intervals in an isolated-perfused failing rabbit heart. The hypothesis of this study was that QT-lengthening compounds will delay ventricular repolarization in the isolated failing heart as it did in the normal isolated hearts of rabbits. It has been reported that rabbit with failing heart has abnormal calcium handling and down regulation of delayed rectifier potassium channel (Rose et al., 2005; Armoundas et al., 2007), which may respond to QT-lengthening compounds in a different manner from normal heart.

In drug safety evaluation, standard repolarization assays and proarrhythmic assessment of new chemical entities may essential. Recently, many disease animal models (i.e., chronic AV block animals, ventricular hypertrophied animals, and heart failure animals) have been introduced for drug safety assessment (Vos et al., 1998; Sugiyama, 2008; Panyasing et al., 2010). However, none of those disease models has been use to test drug effects without the influence of metabolic, humoral, and nervous systems as in the Langendorff preparation. The present study demonstrates that cisapride, dofetilide, and quinidine lengthened QT and QTc(F) interval in both normal and failing hearts. These results agree with the previous studies (Hamlin et al., 2004; Mittelstadt et al., 2006) conducted in isolated rabbit and guinea pig hearts. This result is due to the fact that all three QT-lengthening compounds block Ik, and lengthen the action potential duration by retarding repolarization. It appears from the plots of these intervals against
dose that the lengthening for the failing hearts was rather uniform and parallel to that for the normal hearts, but that lengthening for the normal hearts increased dramatically and inexplicably at the 10 μM concentration of cisapride. It is possible, of course, that at this high concentration, cisapride has a preferential effect on L-type calcium channel (ICa,L), transient outward potassium channel (ITo), IKs or any other channel important in action potential duration (Di Diego et al., 2003; Chiang et al., 2004).

In this study on isolated perfused hearts, cisapride, dofetilide, and quinidine lengthened the RR interval in agreement with previously published results from isolated rabbit and guinea pig hearts (Hamlin et al., 2004; Mittelstadt et al., 2006). In fact RR intervals lengthened identically for both normal and failing hearts at concentrations of cisapride from 0.01 μM to 1 μM; however, RR lengthened less for the failing hearts than for the normals at the 10 μM concentration of cisapride (Fig. 1). At this high concentration it is likely that cisapride also blocks ICa,L channels, which in heart failure, function differently than in normal hearts (Chiang et al., 2004). It is well-known that RR interval depends upon the rate of diastolic depolarization of the SA node, the magnitude of the resting potential, and the threshold potential at which a more rapid rise of the action potential occurs. These factors depend predominantly upon physiology of inward rectifier potassium current (IK1), funny current (IF), and calcium current (ICa). Recent studies in the SA node of rabbit demonstrated that the SA nodal pacemaker cells are composed of both rapid components (IKr) and slow components (IKs) of delayed rectifier potassium current (Lei et al., 2000) and blocking of the IKr suppresses pacemaker activity (Kurata et al., 2003). We do not believe that cisapride affects IK1, IF, and ICa channels, but does affect the hERG channel. Therefore the lengthening of the RR interval (reduction in heart rate) may be explained by blocking of IKr, and retarding repolarization of the SA nodal fibers, and that this effect is overridden by adrenergic mechanisms in the in vivo state.

Fig. 3. Plots of mean values and standard errors of the mean for QTc interval for normal and failing hearts vs concentration of torsadogens or time (vehicle). Asterisk (*p < 0.05) shows where differences differed from baseline in the same group. Tee (τp < 0.05) shows where differences differed between normal hearts and failing hearts at the same concentration. Each value represents the mean ± S.E.M. of an average of one minute of cardiac cycles from a difference rabbit heart recording at the 15th minute of each escalating dose of test articles or equivalent volumes of vehicles.

Fig. 4. Plots of mean values and standard errors of the mean for dP/dt max for normal and failing hearts vs concentration of torsadogens or time (vehicle). Asterisk (*p < 0.05) shows where differences differed from baseline in the same group. Each value represents the mean ± S.E.M. of an average of one minute of cardiac cycles from a difference rabbit heart recording at the 15th minute of each escalating dose of test articles or equivalent volumes of vehicles.
It is clear that failing hearts compared to normal hearts have reduced inotropy and lusitropy during the baseline. In this study, cisapride depressed dP/dt max in normal and failing hearts. These results are consistent with a previous publication in anesthetized dogs, which demonstrated that cisapride decreased dP/dt max at a higher dose (Al-Wabel et al., 2002) due possibly to the blocking effect of cisapride on the ICa,L channel (Chiang et al., 2004). The measures of inotropy (dP/dt max) and lusitropy (dP/dt min) in a Langendorff heart kept at constant preload and afterload should be satisfactory measures of those mechanical properties. However, since failing hearts were slower during the baseline than were normal hearts, the reduction in inotropy may be attributable merely to the slower heart rate (Treppe effect), therefore it would require repeating these studies in hearts with fixed rates to determine if the reduction in inotropy in the failing hearts was due to the slower heart rate or to direct negative inotropy (e.g., altered EC coupling). It is clear that cisapride did not affect either inotropy or lusitropy until the highest concentration at which time, of course, heart rate had decreased precipitously.

Prior publication of our lab on the incidence of TdP in a rabbit model of myocardial failure induced by coronary ligation demonstrated that torsadogens induced TdP in anesthetized rabbits with myocardial failure (Kijtawornrat et al., 2006). However, no concentration of any torsadogen used in this study was able to produce TdP in either normal or failing hearts in Langendorff preparation perfused with Modified Krebs-Henseleit solution. This lack of propensity to develop torsades de pointes in Langendorff preparations when compared to in vivo preparations may arise from the role of autonomic activity or metabolistic factors active in the in vivo preparation (Champeroux et al., 2010; Chinushi et al., 2008).

This study has several limitations. First, we recognize that TdP occurs more prevalently in women than in men (Lehmann et al., 1996), and in female rabbits than in males (Lu et al., 2001; Ruan et al., 2004). However our laboratory has always conducted studies on male rabbits to exclude the role of estrous. Second, we also recognize that TdP occurs more prevalently in a Langendorff perfused with a low K+ or Mg2+ Krebs solution (Delacretaz and Fuhrer, 1999; Dhein et al., 2008; Cheng and Incardona, 2009). However, the present study was aimed to conduct in an isolated heart perfused with a solution that is closely to in vivo preparation in which the sensitivity of failing hearts to develop arrhythmia is higher than normal hearts (Kijtawornrat et al., 2006; Panyasing et al., 2010).

In conclusion, compared to normal hearts, the failing isolated perfused heart manifested clear differences in mechanical properties (inotropy and lusitropy). Langendorff preparations of both normal and failing rabbit hearts are satisfactory for identifying a liability for lengthening of QT or QTc(F) intervals as indicated by all three QT-lengthening compounds prolonged QT and QTc(F) intervals.

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REFERENCES


