Uni- or Bi- Ventricular Hypertrophy and Susceptibility to Drug- induced
Torsades de Pointes

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in
the Graduate School of The Ohio State University

By

Yaowalak Panyasing, D.V.M.

Graduate Program in Veterinary Biosciences

The Ohio State University

2010

Master's Examination Committee:

Robert L. Hamlin, Advisor

Stephen Mark Strauch

Cynthia A. Carnes

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Abstract

Cardiac hypertrophy is an independent risk factor for torsades de pointes (TdP), a polymorphic ventricular tachycardia that is often drug-induced, but that may terminate spontaneously or evolve into ventricular fibrillation and sudden death. People with ventricular hypertrophy or heart failure have an increased risk of drug-induced arrhythmias, therefore this study was designed to determine if right (RVH), left (LVH), or biventricular (BVH) hypertrophy increases susceptibility to drug-induced TdP. Rabbits were separated into 4 groups: control or RVH, LVH, BVH (studied 8 weeks after banding of one or both great arteries). ECGs were recorded continuously after baseline and after rabbits received escalating doses of torsadogens (dofetilide, clofilium and terfenadine) or non-torsadogens (cilobradine, diltiazem and vehicle). The following parameters were measured [RR, PQ, QRS, and QT] or calculated [QTc (F), short term variability of QT interval]. Generally, torsadogenicity for the compounds tested was dofetilide > clofilium > terfenadine, and there was no TdP following cilobradine, diltiazem or vehicle. In general the susceptibility to TdP was RVH > BVH > LVH > control. Rabbits with RVH developed TdP much more prevalently than for those with either LVH or BVH ($p < 0.05$). At low doses of dofetilide, LVH was actually protective. Rabbits with any form of hypertrophy develop prolongation of QT, QTc and increased QT instability.

Rabbits with any form of hypertrophy are more prone to arrhythmia than normals in response to known torsadogens.

Dedication

This document is dedicated to
My parents, Paisan and Meuy Panyasing
And
My Professor, Dr. Robert L. Hamlin

Acknowledgments

I owe my deepest gratitude to my professor, Dr. Robert Hamlin. His encouragement, guidance and support enabled me to complete this thesis.

I am grateful to Dr. Anusak Kijawornrat for his strong support and assistance throughout the development and execution of my thesis.

I would like to thank my committee, Dr. Cynthia A. Carnes and Dr. Stephen M. Strauch for correcting error in my manuscript and their precious comments.

I am grateful to Dr. Carlos del Rio for reviewing and correcting error in my manuscript and his valuable comments.

I would like to thank Mr. David Hamlin for giving me a laboratory to perform my experiments and his other forms of support.

It is a pleasure to thank other laboratory members: Jay Schmidt, Travis Brown, Adriana Pedraza-Toscano, Pedro Vargas-Pinto, Yukie Ueyama and Duane Probst for their great support and assistance.

Last, I would like to thank Dr. Apisit Kittawornrat for his encouragement and inspiration in many ways.

Vita

October 16, 1980.....Born- Bangkok, Thailand

1999- 2005..... Doctor of Veterinary Medicine,
Chulalongkorn University, Bangkok,
Thailand.

2005-2007Clinician, Small Animal Teaching Hospital,
Chulalongkorn University, Thailand

2007- 2009Visiting Scholar, Department of Veterinary
Biosciences, The Ohio State University,
Ohio, USA.

2009- PresentGraduate Research Associate, Department
of Veterinary Biosciences, The Ohio State
University, Ohio, USA.

Publications

Research Publication

1. Kijtawornrat, A., **Panyasing, Y.**, Del Rio, C., and Hamlin, R. L. (2009). Assessment of ECG interval and restitution parameters in the canine model of short QT syndrome. Journal of pharmacological and toxicological methods, 61(3):231-237.

Abstract Publication

1. **Panyasing Y.**, Kijawornrat A., Del Rio C.L., Snedden L., Mongkolmaneeapol T., Schmidt J. and Hamlin RL. (2009). Analysis of dynamic restitution of ECG parameters in the rabbit langendorff heart exposed to drugs that lengthen or abbreviate QT. Journal of pharmacological and toxicological methods, 60(2): 242. Abstr
2. Kijawornrat A., **Panyasing Y.**, Del Rio C.L., Schmidt J., Snedden L., Mongkolmaneeapol T. and Hamlin RL. (2009). Differences in ECG intervals and restitution parameters in the conscious sling-trained canine model of short QT syndrome. Journal of pharmacological and toxicological methods, 60(2): 225. Abstr.
3. Kijawornrat, A., **Panyasing, Y.**, Del Rio, C., Monkolmaneeapol, T., Snedden, L., Schmidth, J. J., and Hamlin, R. L. (2009). Change of dynamic beat- to-beat QT-TQ interval relationship in conscious dog model of short and long QT syndrome. Toxicological sciences, 108 (1):s229. Abstr.
4. Del Rio C.L., Kijawornrat A., **Panyasing Y.**, Snedden L., Probst D., Schmidt J. and Hamlin R.L. (2009). Non-invasive continuous cardiac output assessment via impedance cardiography in a canine model of controlled hemorrhagic shock. Journal of pharmacological and toxicological methods, 60(2): 253. Abstr.

5. Del Rio C.L., Snedden L., Ueyama Y., **Panyasing Y.**, Zijnenberg R., Kijawornrat A., Hamlin R.L. and Muir W.W. (2009). Concentration-dependent changes in ECG-derived indices of repolarization triggered by isoflurane: A study in telemetered beagle dogs. *Journal of pharmacological and toxicological methods*, 60(2): 248-249. Abstr.
6. Kijawornrat, A., **Panyasing, Y.**, Lolly, J.L., Schmidt, J.J., Nishijima, Y., Roach, B.M. and Hamlin, R.L. (2008). Assessment of QTc formulas in conscious dogs with pacing induced heart failure. *Toxicological sciences*, 102(1):s338. Abstr.
7. Hamlin, R. L., Kijawornrat, A., **Panyasing, Y.**, Lolly, J.L., Schmidh, J.J., Snedden, L., and Hamlin, D.M. (2008). Potential impedance of detecting drug- induced alteration in regional distribution of cardiac output. *Toxicological sciences*, 102 (1): S339. Abstr.

Fields of Study

Major Field: Veterinary Biosciences

(Physiology/Pharmacology)

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Introduction

Torsades de pointes (TdP) is a specific polymorphic ventricular tachyarrhythmia that most often terminates spontaneously but may degenerate into ventricular fibrillation and consequent sudden cardiac death ([Shah, 2004](#); [Hamlin, 2006](#)). TdP is frequently drug-induced, and may be caused by either cardiovascular or non-cardiovascular drugs. The potential for drugs to produce TdP is a major concern to both regulatory agencies and pharmaceutical companies.

Currently, prolongation of the QT interval on the ECG is the most commonly used predictor of drug-induced TdP ([Roden, 1998](#)). However, several studies have shown that lengthening of QT interval, alone, is not a good biomarker ([Redfern et al., 2003](#); [Antzelevitch, 2004](#); [Thomsen et al., 2004](#)) since some compounds (e.g., pentobarbital, amiodarone) lengthen QT but do not (or seldom) produce TdP while others do not lengthen the QT interval greatly but produce TdP (e.g., Terfenadine). Increased prevalence of drug-induced TdP has been shown in animal models with myocardial failure ([Kijawornrat et al., 2006](#); [Vos et al, 1995](#)), and other biomarkers such as short-term QT instability, triangulation of the action potential, and reverse-rate-dependence of prolonged repolarization have been suggested to be more predictive of drug-induced TdP than the QT interval ([Hondeghem et al., 2001](#); [Hondeghem & Hoffmann, 2003](#); [Thomsen et al., 2004](#); [Thomsen et al., 2005](#))

It is well known that the prevalence of ventricular arrhythmia or TdP increases in humans with reduced repolarization reserve (Wu et al., 2006; Kahan & Bergfeldt, 2005; Roden & Yang, 2005; Thomsen et al., 2005) particularly when other risk factors are present. These factors may include bradycardia, hypomagnesemia and/or hypokalemia, female sex, congenital long QT syndrome, cardiomyopathy and pathologic electrical remodeling (Antzelevitch et al., 1999; El-Sherif & Turitto, 1999; Sipido et al., 2000).

According to the updated Heart Disease and Stroke Statistics (AHA, 2009), an estimated 80 million American adults have one or more type of cardiovascular diseases, of whom 73.6 million are hypertensive or pre-hypertensive. These people may possess a higher risk for drug-induced TdP.

Hypertension or pressure-overload causes development of hypertrophy which may lead to maladaptive compensation (Berenji et al., 2005; Kang, 2006). Cardiac hypertrophy is associated with QT interval prolongation and enhanced susceptibility to torsadogenic compounds (Kozhevnikov et al., 2002; Schoenmakers et al., 2003; Swynghedauw et al., 2003). Reduction of ion currents in hypertrophied heart such as I_{to} , I_{Ks} and I_{Kr} may result in prolongation of cardiac action potential increasing the arrhythmogenicity (Tomaselli & Marban, 1999; Kang, 2006).

However, there is no comparative information on the susceptibility to drug-induced TdP with the various types of ventricular hypertrophy. We used rabbit models of right (RVH), left (LVH), or biventricular (BVH) hypertrophy to evaluate arrhythmia susceptibility, and specifically susceptibility to drug-induced TdP.

The hypotheses of this study were: (1) Rabbits with any form of hypertrophy have longer QT and QTc intervals than normal rabbits; (2) Rabbits with any form of hypertrophy are more likely to develop TdP in response to known torsadogens than rabbits with normal hearts; (3) the incidence of TdP is greatest with BVH, next greatest with LVH, next greatest with RVH, and least in normals (controls).

Materials and Methods

Approval/Animal

This study was conducted after approval of the Institutional Animal Care and Use Committee (IACUC) of QTest labs and in compliance with USDA regulations. A total of 48 New Zealand White, young-mature female rabbits weighing 2.5 to 3.8 kg were used in this study.

Rabbits were selected for this study because they possess a spectrum of ion channels specific for ventricular repolarization that mimic, closely, the spectrum in man ([Gintant, 2008](#)), because we have considerable experience studying TdP in rabbits with heart failure ([Kijawornrat et al., 2006](#)), and because they usually have prominent, easily defined QT intervals.

Surgical procedure for ventricular hypertrophy rabbit model

Rabbits were anesthetized IM with 35 mg/kg ketamine and 5 mg/kg xylazine and surgical anesthesia was maintained with 0.8-1.5 % isoflurane delivered by mask. A flow rate of 400-600 ml/min of 100% oxygen was applied through the mask. Rabbits were placed in dorsal recumbency, the ventral aspect of the thorax from manubrium to xiphoid was

denuded and prepared with aseptic technique. ECG lead II was monitored during surgery. The incision line was infiltrated with 2% lidocaine. A midline incision was made starting from the sternum approximately 3-4 cm in length. The sternal cartilage was split and kept open with self-retractors. The pericardial sac was cut to access the pulmonary artery or ascending aorta. Either pulmonary artery or aorta was gently separated and elevated with right angle forceps. A sterile polyethylene tube approximately 50% of the diameter of the great artery was sutured to the artery, then, when the tube was withdrawn, the vessel was left narrowed approximately 50%. The pericardial sac was left open. Muscle layers and the sternum were sutured with 3-0 monofilament polydioxanone suture. Skin was stapled. Enrofloxacin 12 mg/kg BID IM was given preoperatively and for 2 days after surgery. Rabbits also received 0.03 mg/kg BID buprenorphine intramuscularly for 2 days postoperatively. Rabbits were kept for 8 weeks post surgery to allow for development of hypertrophy.

Experimental protocol

Rabbits were separated into 4 groups: (I) control (age matched, sham operated, $n=8$), (II) right ventricular hypertrophy (RVH, $n=9$), (III) left ventricular hypertrophy (LVH, $n=9$), and (IV) biventricular hypertrophy (BVH, $n=10$). Animals were studied 8 weeks after banding of one or both great arteries, when hypertrophy was documented by echocardiography. For each drug exposure, rabbits were anesthetized with a single IM dose of ketamine (35 mg/kg) and xylazine (5 mg/kg) that rendered the rabbits motionless

and free from discomfort for the 1 h required. ECG lead II was used to monitor rhythm and analyze drug effects on electrophysiological parameters (HR, RR, PQ, QRS, and QT intervals). ECGs were recorded continuously on an IOX2 acquisition system. All groups were subjected to 8 to 14 weeks of banding. They were given test articles (dofetilide, clofilium, terfenadine, cilobradine, diltiazem, or vehicle) in random order with 1 week washout in-between. The dose of each torsadogen was selected from rabbit studies conducted previously in our laboratories or others ([Kijawornrat et al., 2006](#); [Batey & Coker, 2002](#)). The marginal ear vein was cannulated with a 24-gauge introducer for intravenous administration of drugs. An infusion pump (KD scientific, Holliston, MA) was set to control the volume and rate of drug administration. Stabilization time was at least 10 min before infusion. Each dose was infused over a period of 10 min, with a 5 minute interval between doses. The infusion was stopped at the end of the experiment or as soon as TdP occurred. Magnesium sulfate (~50 mg/kg) was administered to animals with TdP at a rate of 5 mg/kg/min in order to terminate the arrhythmia. The infusion was not continued to a higher dose when TdP occurred at a lower dose. We did not assume that because TdP occurred at a lower dose it would have occurred at all higher doses.

Heart weight-body weight ratio was calculated after the last study to evaluate cardiac hypertrophy.

Electrocardiographic assessment

Standard electrocardiogram (ECGs) lead II was obtained before and 8 weeks after surgery in order to assess changes in rabbits with hypertrophic hearts (IOX2 version 2.2.17.19; EMKA Technologies, Falls Church, VA). The following parameters were measured (HR, RR, PQ, QRS, and QT) or calculated, QTc (F) ([Fridericia, 1920](#)), short-term variability of QT interval ([Thomsen et al., 2004](#)). ECGs were recorded continuously during dosing to search for arrhythmias in response to known torsadogens and non-torsadogens.

Echocardiographic assessment

Echocardiograms (Mega ES Biosound ESAOTE Inc, Indianapolis, Indiana) were obtained after sedation with 15 mg/kg ketamine and 3 mg/kg xylazine using a 7.5 MHz probe to obtain a right parasternal, 2D image of the ventricles. The cursor was set from the 2D image to obtain an M-mode for measuring ventricular wall thickness and LV ejection fraction. The following parameters were assessed before and 8 weeks after surgery: interventricular septum diastolic dimension (IVSd), interventricular septum systolic dimension (IVSs), left ventricular internal diastolic diameter (LVIDd), left ventricular internal systolic diameter (LVIDs), left ventricular posterior wall diastolic dimension (LVPWd), left ventricular posterior wall systolic dimension (LVPWs), ejection fraction (EF), and fractional shortening (FS).

Drugs

Dofetilide (a blocker of I_{Kr}) was dissolved in a 0.9% NaCl solution acidified with 0.1M hydrochloric acid, at a stock concentration of 0.1 mg/ml. The escalating doses of dofetilide were 0.005, 0.01, and 0.02 mg/kg/10 min. Clofilium tosylate (a blocker of I_{Kr}) (Sigma, St Louis, MO) was dissolved in deionized water to a final concentration of 1 mg/ml. The escalating doses of clofilium were 0.1, 0.2, and 0.4 mg/kg/10 min.

Terfenadine (a blocker of I_K , I_{Na} , I_{CaL}) (Sigma, St Louis, MO) was dissolved in 20 % polyethylene glycol 400, acidified with 0.1M hydrochloric acid, and sonicated at room temperature. It was dissolved in 5% dextrose solution to make a final concentration of 3.54 mg/ml ([Batey & Coker, 2002](#)). The escalating doses of terfenadine were 0.035, 0.12, and 0.36 mg/kg/min. We prepared a vehicle for terfenadine because we had never used it in previous studies and did not know what effects that vehicle might have on electrophysiology. As in previous studies by others ([Batey&Coker, 2002](#)) this terfenadine vehicle served as a control. The vehicle for terfenadine was prepared by dissolving all substances used for the terfenadine solution, and the volume given was the same as for terfenadine. Cilobradine (a blocker of I_f) was dissolved in deionized water, to make a final concentration of 1 mg/ml. This solution was protected from light by wrapping with foil. The escalating doses of cilobradine were 0.5, 1.0, and 1.5 mg/kg/10 min. Diltiazem HCl (a blocker of I_{CaL}) (25 mg/ml) was diluted with 0.9% w/v NaCl solution to form a stock solution at 0.5 mg/ml. The escalating doses of diltiazem were 0.1, 0.3, and 0.5 mg/kg/10min.

Data analysis

ECGs were analyzed offline by means of computer-assisted pattern recognition software (ECG Auto Version 2.5.1.16; EMKA Technologies, Falls Church, VA). HR, RR, PQ, QRS, and QT were measured and QTc(F) were calculated for 1 min at steady state of baseline and after the end of each concentration for test articles or vehicle. Short term QT variability/instability (STV) was calculated as the mean orthogonal distance (D) from the points comprising the cloud to the diagonal ([Thomsen et al., 2004](#)). The difference in STV attributable to hypertrophy was measured as the difference between STV at baseline and at 8 weeks following banding.

Statistic

Data were expressed as mean $\pm$ standard error of the mean (SEM). Values for electrocardiographic (RR, PQ, QRS, QT and QTc,) and echocardiographic (IVSd, IVSs, LVIDd, LVIDs, LVPWd and LVPWs) parameters were compared among groups (control vs. RVH vs. LVH vs. BVH) 8 weeks after surgery; inter-group differences on the means were evaluated by two- way ANOVA, and if indicated by a significant F-statistic, pair-wise means were compared by Tukey post-hoc analyses. Meanwhile, intra-group (group by group) values for parameters of QT instability were compared over time (i.e., between 8 weeks and baseline) using a paired Student's t-test. On the other hand, the incidence of

TdP was compared among groups via a Fisher exact test. In all cases, $p < 0.05$ was considered (a priori) as statistically significant.

Results

A total of 48 rabbits were operated on for this study. Because of surgical and post-operative mortality (always occurring during the first post-operative week), rabbits were entered successfully 9 out of 12 with RVH, 9 out of 10 with LVH, and 10 out of 17 with BVH. Thus death rates were 25% for RVH, 10% for LVH, and 41% for BVH. Only 1 rabbit died during surgery.

Ventricular Hypertrophy:

Electrical remodeling

Comparisons between electrocardiographic indices were made between baseline and 8 weeks in each group, between the control animals (sham-operated) and the groups with hypertrophy (banding) at 8 weeks after surgery; table 1 summarizes these results. In short, QTc lengthened in all hypertrophic groups, while the uncorrected QT-interval lengthened only in the RVH group. No other ECG changes achieved significance; however, the QRS duration tended to be greater 8 weeks after banding in rabbits with any form of hypertrophy (42.3 ± 1.7 ms for RVH, 40.5 ± 1.6 ms for LVH, 42.5 ± 2.3 ms for BVH) when compared to controls (39.2 ± 1.5 ms). No differences in parameters at 8 weeks occurred among the hypertrophic groups.

In addition, the short term QT instability in ms (Table 2), an index thought to reflect heterogeneity of ventricular repolarization and susceptibility to induced arrhythmias was significantly increased in hypertrophic animals when compared to control group at 8 weeks after banding and when compared to baseline in each group. Representative individual examples of Poincare' plots from group of control, RVH, LVH, and BVH representing instability of QT are shown in Figures 1, 2, 3, 4 respectively. Briefly, the degree of “openness” of the cluster (cloud) of points represents short-term QT variability/instability.

Table 1: Measurements of electrocardiograms before and after 8 weeks after sham-operation or banding of pulmonary artery, ascending aorta, or both vessels.

Electrocardiograms		RR (ms)	PQ (ms)	QRS (ms)	QT (ms)	QTc (ms)
Control(n=8)	BL	306.4±22.8	73.3±3.2	37.6±2.0	155.2±5.3	227.1±6.2
	8 WKs	321.3±12.5	80.9±4.0	39.2±1.5	157.4±6.0	229.9±5.6
RVH(n=9)	BL	353.7±15.3	74.7±2.2	38.2±1.6	159.0±4.1	230.9±4.3
	8 WKs	354.5±11.2	75.2±2.8	42.3±1.7	176.0±4.8* [#]	249.2±5.7* [#]
LVH(n=9)	BL	348.2±13.0	80.4±3.1	39.0±2.6	158.2±4.2	226.5±5.7
	8 WKs	311.9±10.7	76.9±1.8	40.5±1.6	168.8±5.3	240.6±4.5* [#]
BVH(n=10)	BL	326.1±18.9	75.5±4.5	40.7±1.8	156.6±5.5	228.3±3.0
	8 WKs	317.5±6.4	77.1±2.9	42.5±2.3	165.2±1.8	242.3±1.5* [#]

Values are shown as mean ± SEM. RVH = Right ventricular hypertrophy, LVH = Left ventricular hypertrophy, BVH = Biventricular hypertrophy. * Indicates significance $p < 0.05$ compared to control group. [#] Indicates statistical significance ($p < 0.05$) when compared to the baseline in the same group. Notice: QTc prolonged in all hypertrophy models.

Table 2: Short-term QT instability (ms) compared between baseline and 8 weeks in control and hypertrophic hearts.

QT instability	Control (<i>n</i> =8)	RVH (<i>n</i> =9)	LVH (<i>n</i> =9)	BVH (<i>n</i> =10)
Baseline	0.24±0.07	0.30 ± 0.05	0.34±0.06	0.29±0.05
8 weeks	0.31±0.05	2.57± 0.11 ^{*#‡}	1.59 ± 0.09 ^{*#}	1.67 ± 0.14 ^{*#}

*Indicates statistical significance ($p < 0.05$) when compared to control at 8 weeks; [#] Indicates statistical significance ($p < 0.05$) when compared to the baseline in the same group. [‡] This indicates statistical significance ($p < 0.05$) when compared among hypertrophic groups.

Figure 1: Examples of beat-to-beat Poincaré plot of QT intervals in one cycle (QT_n) to its subsequent cycle (QT_{n+1}) in the anesthetized rabbit with a normal heart .

Plots were calculated for 30 cycles from each animal evaluated at baseline and 8 weeks after banding. Numbers represent root mean squares of distances points are from the diagonal. Short term QT instabilities in 8 rabbits without hypertrophy before and after banding were 0.21 ± 0.04 and 0.30 ± 0.07 respectively.

Figure 2: Examples of beat-to-beat Poincaré plot of QT intervals in one cycle (QT_n) to its subsequent cycle (QT_{n+1}) in the anesthetized rabbit with right ventricular hypertrophy.

Plots were calculated for 30 cycles from each animal evaluated at baseline and 8 weeks after banding. Numbers represent root mean squares of distances points are from the diagonal. The short-term variability is significantly greater after banding. Short term QT instabilities in 9 rabbits with right ventricular hypertrophy before and after were 0.27 ± 0.06 and 2.64 ± 0.09 respectively.

Figure 3: Examples of beat-to-beat Poincaré plot of QT intervals in one cycle (QT_n) to its subsequent cycle (QT_{n+1}) in the anesthetized rabbit with left ventricular hypertrophy.

Plots were calculated for 30 cycles from each animal evaluated at baseline and 8 weeks after banding. Numbers represent root mean squares of distances points are from the diagonal. Short-term variability is much greater after banding. Short term QT instabilities in 8 rabbits with left ventricular hypertrophy before and after banding were 0.38 ± 0.06 and 1.59 ± 0.11 respectively.

Figure 4: Examples of beat-to-beat Poincaré plot of QT intervals in one cycle (QT_n) to its subsequent cycle (QT_{n+1}) in the anesthetized rabbit with biventricular hypertrophy.

Plots were calculated for 30 cycles from each animal evaluated at baseline and 8 weeks after banding. Numbers represent root mean squares of distances points are from the diagonal. Short-term variability is much greater after banding. Short term QT instabilities in 10 rabbits with biventricular hypertrophy before and after banding were 0.24 ± 0.05 and 1.76 ± 0.14 respectively.

Geometrical/Functional Remodeling

Left-ventricular echocardiographic interrogation 8-weeks after surgery demonstrated significant ventricular remodeling in animals with aortic banding (i.e., both LVH and BVH groups), while no notable differences were observed between normal controls and pulmonary artery banded rabbits. For instance, the septal and free walls (IVSd and LVPWd, respectively) thickened for both the LVH and the BVH groups. In addition, animals with exclusive left ventricular hypertrophy also showed signs of chamber dilatation (increased LVIDd and LVIDs dimensions). Table 3 summarizes the echocardiographic findings/parameters for all groups. In all cases, ventricular function appeared to be preserved.

Table 3: Measurements from two-dimensional, short-axis echocardiograms comparing before and after 8 weeks after sham-operation or banding of pulmonary artery, ascending aorta, or both vessels.

Echocardiogram		Control	RVH	LVH	BVH
Parameters		(n=8)	(n=9)	n=8)	(n=10)
IVSd (cm)	BL	0.22±0.02	0.27±0.02	0.23±0.02	0.25±0.01
	8 WKs	0.24±0.01	0.26±0.03	0.29±0.03* [#]	0.28±0.01* [#]
IVSs (cm)	BL	0.30±0.01	0.32±0.04	0.28±0.01	0.31±0.02
	8 WKs	0.29±0.01	0.31±0.03	0.35±0.03* [#]	0.33±0.03
LVIDd (cm)	BL	1.31±0.06	1.39±0.18	1.40±0.06	1.35±0.07
	8 WKs	1.38±0.05	1.47±0.15	1.78±0.15* [#]	1.57±0.06
LVIDs (cm)	BL	0.98±0.03	1.07±0.04	1.04±0.02	0.97±0.04
	8 WKs	1.01±0.04	1.02±0.07	1.26±0.07* [#]	1.13±0.06
LVPWd (cm)	BL	0.23±0.01	0.29±0.01	0.24±0.01	0.26±0.01
	8 WKs	0.26±0.01	0.30±0.02	0.35±0.02* [#]	0.34±0.01* [#]
LVPWs (cm)	BL	0.40±0.02	0.42±0.03	0.37±0.01	0.36±0.02
	8 WKs	0.40±0.01	0.41±0.02	0.48±0.02* [#]	0.43±0.05
EF (%)	BL	56.2±2.10	63.40±4.18	58.60±2.65	58.60±2.30
	8 WKs	56.8±1.61	59.83±3.79	58.85±3.79	58.03±2.48
FS (%)	BL	26.40±1.12	31.60±2.98	28.60±2.10	28.40±1.01
	8 WKs	27.18±1.03	29.48±2.79	29.17±2.79	28.22±1.53

Values are shown as mean ± SEM. IVSd = Interventricular septum, diastolic dimension; IVSs = Interventricular septum, systolic dimension; LVIDd = Left ventricular internal, diastolic dimension; LVIDs = Left ventricular internal, systolic dimension;

LVPWd =Left ventricular posterior wall, diastolic dimension; LVPWs = Left ventricular posterior wall, systolic dimension; EF = Ejection fraction; FS = Fractional shortening. * indicates significance $p < 0.05$ versus control group. # indicates significance $p < 0.05$ versus baseline in each group.

Hearts from rabbits with all forms of hypertrophy were significantly heavier than hearts from the control rabbits. The heart weight-body weight ratio increased significantly ($p < 0.05$) in all hypertrophic groups compared to controls (Table 4). No differences in parameters at 8 weeks occurred among hypertrophic groups.

Table 4: The heart weight-body weight ratio in control and hypertrophic rabbits.

HW/BW (g/kg)	Control	RVH	LVH	BVH
	2.24±0.04	3.26±0.11*	3.33±0.07*	3.88±0.14*

* Indicates significance $p < 0.05$ when compared to control group.

Incidence of Drug-induced Torsades de Pointes

Figure 5 shows the incidence of TdP in each of 4 groups (control, RVH, LVH and BVH) given vehicle or any of the 5 active drugs. No groups developed an increase in prevalence of TdP in response to terfenadine, cilobradine, diltiazem, or vehicle. Rabbits with RVH developed TdP more prevalently than for those with either LVH or BVH in response to escalating doses of both dofetilide and clofilium. Figure 6, 7 shows that TdP developed more prevalently in rabbits with RVH in a general dose-response pattern for dofetilide, however it became significant only at the high dose. A similar pattern developed for the group with BVH, but did not achieve statistical significance. For the group with LVH, the incidence of TdP was not observed for the low and medium doses of dofetilide, and the apparent increase for the high dose did not achieve significance. Figure 8, 9 shows comparable data only for response to clofilium. At the low dose, clofilium did not produce TdP in any group. At the mid-dose, clofilium produced a significant increase in the group with RVH, a non-significant increase in the group with BVH, and no increase in the group with LVH. At the high dose of clofilium, TdP occurred in all groups, that with BVH appeared to be the greatest, but none achieved statistical significance. Whereas terfenadine did not induce TdP in any group, it induced rapid ventricular tachycardia in 2 rabbits that died without developing ventricular fibrillation.

Figure 5: Effects of torsadogenic compounds (dofetilide, clofilium, and terfenadine) and non-torsadogenic compounds (vehicle, cilobradine, and diltiazem) on the incidence of torsades de pointes (TdP) in anesthetized rabbits with and without ventricular hypertrophy.

* Indicates significance $p < 0.05$, ** $p < 0.01$ when compared to control group.

Figure 6: Effects of escalating doses of dofetilide on frequency of TdP (%) on rabbits with or without hypertrophied hearts.

* Indicates significance $p < 0.01$. Notice: RVH produced the highest (and significant) incidence of TdP at the highest dose. At the medium dose TdP occurred in both RVH and BVH, but not LVH. n represents number of animal per group; Control, $n = 8$; RVH, $n = 9$; LVH, $n = 9$; BVH, $n = 10$.

Figure 7: Cumulative incidences of escalating doses of dofetilide on frequency of TdP (%) on rabbits with or without hypertrophied hearts.

n represents number of animal per group; Control, $n = 8$; RVH, $n = 9$; LVH, $n = 9$; BVH, $n = 10$.

Figure 8: Effects of escalating doses of clofilium on incidence of TdP (%) in rabbits with or without hypertrophic hearts.

* Indicates $p < 0.01$. Notice: clofilium induced high (significant) incidence of TdP at medium dose in RVH group, whereas it induced TdP only at the high dose in the LVH group. This is similar to the response to dofetilide. n represents number of animal per group; Control, $n = 8$; RVH, $n = 7$; LVH, $n = 8$; BVH, $n = 9$.

Figure 9: Cumulative incidence of escalating doses of clofilium on incidence of TdP (%) in rabbits with or without hypertrophic hearts.

n represents number of animal per group; Control, $n = 8$; RVH, $n = 7$; LVH, $n = 8$; BVH, $n = 9$.

Two-hundredths (0.02) of a mg/kg of dofetilide induces only QT prolongation in control group. In contrast, at the same dose, dofetilide induced TdP in all groups of hypertrophy (Figure 10)

Figure 10: Examples of bipolar, transthoracic ECGs from a rabbit given an IV of 0.02 mg/kg dofetilide.

Rabbits (from top panel to bottom panel), are: normal, RVH, LVH and BVH. Notice that rabbits with hypertrophic hearts developed TdP, but that the normal rabbit had a dramatically prolonged QT, but did not develop TdP.

Drug-induced Changes in QTc

Dofetilide, clofilium, and terfenadine (Figure 11, 12, 13) appeared to lengthen QTc in a dose-response manner for all groups, albeit less pronounced between the mid and high doses for the group with RVH for dofetilide. Some of these differences achieved statistical significance, but all appeared qualitatively similar. Cilobradine appeared to lengthen QTc in a general dose-response pattern in all groups (Figure 14). The responses to diltiazem and vehicle were slight and insignificant (Figure 15, 16).

Figure 11: Plots of percent change of QTc (ms) from baseline versus each dose (mg/kg) of dofetilide for rabbits with normal or hypertrophic hearts.

*Indicates statistical significance ($p < 0.05$) when compared to baseline in the same group; # indicates statistical significance ($p < 0.05$) when compared to the control group at the same dose. n represents number of animal per group; Control, $n = 8$; RVH, $n = 9$; LVH, $n = 9$; BVH, $n = 10$. QTc (BL): Control= 225.8 ± 5.0 ms; RVH= 240.4 ± 3.1 ms; LVH= 237.9 ± 2.3 ms; BVH= 238.7 ± 5.6 ms.

Figure 12: Plots of percent change of QTc (ms) from baseline versus each dose (mg/kg) of clofilium for rabbits with normal or hypertrophic hearts.

*Indicates statistical significance ($p < 0.05$) when compared to baseline in the same group; # indicates statistical significance ($p < 0.05$) when compared to the control group at the same dose. n represents number of animal per group; Control, $n = 8$; RVH, $n = 7$; LVH, $n = 8$; BVH, $n = 9$. QTc (BL): Control= 231.1 ± 3.8 ms; RVH= 237.4 ± 4.5 ms; LVH= 239.3 ± 4.4 ms; BVH= 239.2 ± 7.8 ms.

Figure 13: Plots of percent change of QTc (ms) from baseline versus each dose (mg/kg) of terfenadine for rabbits with normal or hypertrophic hearts.

*Indicates statistical significance ($p < 0.05$) when compared to baseline in the same group; # indicates statistical significance ($p < 0.05$) when compared to the control group at the same dose. n represents number of animal per group; Control, $n = 8$; RVH, $n = 7$; LVH, $n = 9$; BVH, $n = 8$. QTc (BL): Control= 224.8 ± 9.4 ms; RVH= 247.4 ± 11.4 ms; LVH= 237.7 ± 5.1 ms; BVH= 238.1 ± 6.7 ms.

Figure 14: Plots of percent change of QTc (ms) from baseline versus each dose (mg/kg) of cilobradine for rabbits with normal or hypertrophic hearts.

*Indicates statistical significance ($p < 0.05$) when compared to baseline in the same group; # indicates statistical significance ($p < 0.05$) when compared to the control group at the same dose. n represents number of animal per group; Control, $n = 8$; RVH, $n = 9$; LVH, $n = 9$; BVH, $n = 10$. QTc (BL): Control= 225.4 ± 2.1 ms; RVH= 240.5 ± 5.1 ms; LVH= 238.7 ± 4.5 ms; BVH= 242.3 ± 4.8 ms.

Figure 15: Plots of percent change of QTc (ms) from baseline versus each dose (mg/kg) of diltiazem for rabbits with normal or hypertrophic hearts.

*Indicates statistical significance ($p < 0.05$) when compared to baseline in the same group; # indicates statistical significance ($p < 0.05$) when compared to the control group at the same dose. n represents number of animal per group; Control, $n = 8$; RVH, $n = 6$; LVH, $n = 9$; BVH, $n = 9$. QTc (BL): Control= 231.6 ± 12 ms; RVH= 247.8 ± 13.4 ms; LVH= 239.9 ± 4.1 ms; BVH= 244.2 ± 8.3 ms.

Figure 16: Plots of percent change of QTc (ms) from baseline versus each dose (mg/kg) of vehicle for rabbits with normal or hypertrophic hearts.

*Indicates statistical significance ($p < 0.05$) when compared to baseline in the same group; # indicates statistical significance ($p < 0.05$) when compared to the control group at the same dose. n represents number of animal per group; Control, $n = 8$; RVH, $n = 9$; LVH, $n = 9$; BVH, $n = 9$. QTc (BL): Control= 229.7 ± 5.8 ms; RVH= 251.7 ± 7.7 ms; LVH= 239.2 ± 5.3 ms; BVH= 246.7 ± 7.9 ms.

Drugs -induced changes in ECG intervals

Terfenadine failed to induce TdP in rabbits with or without hypertrophy; however, it prolonged significantly both PQ interval and QRS duration in rabbits with hypertrophy at high dose (Figure 17, 18). Cilobradine increased significantly RR interval in all groups (Figure 19). Diltiazem tended to increase PQ interval in a general dose-response pattern in all groups (Figure 20). No ECG parameters including RR, PQ, QRS, QT and QTc changed in response to vehicle administration in all groups.

Figure 17: PQ intervals responding to escalating doses of terfenadine in rabbits with or without hypertrophy.

*Indicates statistical significance ($p < 0.05$) when compared to baseline in the same group; # indicates statistical significance ($p < 0.05$) when compared to the control group at the same dose. n represents number of animal per group; Control, $n = 8$; RVH, $n = 7$; LVH, $n = 9$; BVH, $n = 8$.

Figure 18: QRS durations responding to escalating doses of terfenadine in rabbits with or without hypertrophy.

*Indicates statistical significance ($p < 0.05$) when compared to baseline in the same group; # indicates statistical significance ($p < 0.05$) when compared to the control group at the same dose. n represents number of animal per group; Control, $n = 8$; RVH, $n = 7$; LVH, $n = 9$; BVH, $n = 8$.

Figure 19: RR intervals responding to escalating doses of cilobradine in rabbits with or without hypertrophy.

*Indicates statistical significance ($p < 0.05$) when compared to baseline in the same group. As can be seen since at low dose RR interval prolongs significantly in all rabbits with or without hypertrophy. There are no difference between group at any concentration. n represents number of animal per group; Control, $n = 8$; RVH, $n = 9$; LVH, $n = 9$; BVH, $n = 10$.

Figure 20: PQ intervals responding to escalating doses of diltiazem in rabbits with or without hypertrophy.

n represents number of animal per group; Control, $n = 8$; RVH, $n = 6$; LVH, $n = 9$; BVH, $n = 9$.

Discussion

Animal studies have proven useful to determine if a drug might produce TdP. However instead of using TdP as an end-point of study, lengthening of QTc is used more commonly. Because most instances of TdP in humans occur in the presence of secondary risk factors (e.g., heart failure, ventricular hypertrophy, diabetes, obesity, hypokalemia/hypomagnesaemia), we hypothesized in this study that, compared to normal rabbits, rabbits with uni- or biventricular hypertrophy would (1) develop greater lengthening of QTc, (2) would develop higher incidence of TdP, and (3) that TdP would decrease in incidence from BVH>LVH>RVH>normal. Hypotheses (1) and (2) were accepted, and hypothesis (3) was rejected by this study.

We selected to investigate the torsadogenic potential of 6 test articles, 3 known torsadogens and 3 thought to possess minimal to no torsadogenic potential. This study validates that classification, since none of the sham-operated rabbits developed TdP in response to the 3 negative controls, whereas all developed TdP in response to the known torsadogens.

By 8 weeks after surgery, rabbits with any form of banding had greater lengthening of both absolute (QT) and rate-corrected (QTc) QT-interval values when compared to sham-operated rabbits.

Although duration of QRS for banded rabbits tended to be greater than for sham-operated rabbits, the differences did not achieve significance. No doubt, had a larger number of rabbits been studied, the differences would have achieved statistical significance. However it is known that hypertrophy results in lengthening of QRS duration (due to increased ventricular mass ([Okin et al., 1995](#)) without a change in conduction velocity ([Toyoshima et al., 1982](#); [Cookin et al., 1998](#)). Hypertrophy also alters the Na/Ca exchanger, I_{CaL} , I_{K1} , I_{to} ([Wolk, 2000](#)) and I_{Kr} ([Swynghedauw et al, 2003](#); [Kang, 2006](#)) in a manner to lengthen ventricular repolarization. Therefore we confirmed that rabbits with hypertrophy of any form developed both lengthening of QTc (hypothesis 1) and TdP (hypothesis 2).

We observed that the more QTc lengthened (Table 1) or the greater the increase in QT instability (Table 2), the greater was the tendency to develop TdP. Therefore, both QTc lengthening and QT instability ([Hondeghem et al., 2001](#); [Thomsen et al., 2004](#); [Thomsen et al., 2006](#)) predicted TdP. We observed that the incidence of TdP occurred in the following order: RVH > BVH > LVH > sham-operated (normal), thus rejecting hypothesis (3). In fact it appears that LVH protected against development of TdP when compared to RVH or BVH

These results agree with others that both lengthening of QT and increased short-term QT instability should be considered in determining if a given test article is torsadogenic ([Hondeghem et al., 2001](#); [Thomsen et al., 2006](#)). Our results agree with the concept of TRIad (triangulation of the action potential, reverse use dependence, and

instability) (Hondeghem et al., 2001), and dispersion of ventricular repolarization (Belardinelli et al., 2003) for predicting torsadogenic potential.

Clofilium is known to prolong the QT interval, and to produce TdP (Johna et al., 1998; Li et al., 1996; Batey & Coker, 2002). In agreement with those results, clofilium provoked TdP in animals with any form of hypertrophy, albeit to a lesser degree, in sham-operated controls; notably, the incidence of clofilium-induced TdP was significantly greater in the presence of either RVH or BVH (but not in LVH). Clofilium acts as an I_{Kr} antagonist, but can also decrease conductances over L-type Ca^{2+} and Na^{+} channels (Li et al., 1996). In this study, neither PQ nor QRS durations changed following clofilium administration, thus its torsadogenicity could be attributed primarily to the physiological effects of clofilium to I_{Kr} blockade.

Similarly, dofetilide, a potent and selective I_{Kr} blocker generally used as a positive control to induce TdP (Kozhevnikov et al., 2002; Schoenmakers et al., 2003), triggered TdP in 100% of rabbits with RVH, and to a lesser degree in animals with BVH or LVH (no TdP was seen in normal animals)

Terfenadine did not induce TdP in this study; however 2 rabbits receiving the highest dose died from rapid ventricular tachycardia (in RVH and LVH). Terfenadine at high concentrations also prolonged the PQ and QRS durations significantly. These observations are in accord with a study (Batey & Coker (2002) showing that rabbits receiving terfenadine failed to develop TdP but had significant prolongation of both PR (PQ) interval and QRS duration. In our study terfenadine lengthened QTc slightly and significantly, but in the study of Batey and Coker QTc lengthened slightly and

insignificantly. It is known that terfenadine blocks multiple channels in cardiac myocytes (I_{Na} , I_{Ca-L} , I_{Kr} , I_{Ks} , I_{to}) (Crumb et al., 1995; Liu et al., 1997; Lu & Wang, 1999; Ming & Nordin, 1995), and interacts with depolarized and injured tissue more potently than with normal tissue (Liu et al., 1997).

Taken together, these observations (i.e., the incidence of TdP in response to the I_{Kr} blockers dofetilide and clofilium) strongly suggest that rabbits with induced hypertrophy are more susceptible to torsadogens because of altered/remodeled repolarizing K^+ ion channel physiology, particularly affecting I_{Kr} channels. Interestingly, animals with only right ventricular hypertrophy, showed the highest sensitivity to I_{Kr} -blockade induced TdP, suggesting a preferential remodeling of this channel when compared to other forms of hypertrophy. Notably, in dogs with biventricular hypertrophy induced by chronic AV block, I_{Kr} was shown to be down regulated only in the right ventricle and this change was considered to be related with TdP (Volders et al., 1999). Moreover, Gillis et al., (1998) reported that I_{Kr} did not change in rabbits with induced LVH. These reports are consistent with the present findings, which highlight right-ventricular remodeling in the susceptibility to torsadogens.

In addition, there are other possible mechanisms, leading to increase in automaticity and/or reentry, by which rabbits with RVH could present a higher sensitivity to torsadogens.

For instance, banding of a great artery leads to dilatation and hypertrophy of the chamber from which the artery arises. This imposes increased myocardial tension, which in turn may result in mechanoelectric feedback (MEF) (Chen et al., 2004) mediated over

stretch-activated channels (SAC) (Craelius et al., 1988; Seo et al., 2010). It is known that moderate (<15%) and spatially heterogeneous stretches are more arrhythmogenic than greater (20%) or spatially homogeneous stretches (Seo et al., 2010). The increased stretches result in both prolongation of the action potential duration (consistent with increased QT duration/instability) and in EADs (known to be a principal cause of TdP) (Wang et al., 2005). We believe that because the geometry of the crescentic right ventricle is so different from that of the elliptical left ventricle (Rodriguez et al., 2005; Katz, 2000), it is plausible that the stretches imposed on the right ventricle with pulmonary artery banding fulfill the criteria for greater SAC than might occur with banding of the aorta for the left ventricle. Furthermore, because ventricles are interdependent (i.e., sharing the interventricular septum), it is plausible that with BVH the hypertrophic left ventricle alters (either decreasing or increasing) stretch in the right ventricle in a manner that reduces SAC.

In addition, rabbits with RVH may have a relatively greater increase in the ratio of “M” fibers to myocytes; although their existence remains controversial, “M” fibers are thought to be the loci of EAD’s that initiate TdP (Dilaveris & Stefanadis, 2005). Furthermore, in one study, the density of I_f in the left ventricle was found to be significantly higher in hypertrophied myocytes (Velasco et al., 2006) and in cells from spontaneously hypertensive rats (SHR) (Cerbai et al., 1994). The up-regulation of I_f may contribute to accelerated diastolic depolarization and increased automaticity.

On the other hand, it is known that the density of I_{K1} channels in the right ventricle is less than for the left ventricle (Warren et al., 2003). As mentioned above, with

right ventricular hypertrophy but not with left ventricular hypertrophy, there is down-regulation of I_{K1} . Potassium ion flux out of the myocyte through the I_{K1} channel hyperpolarizes the membrane, thus the RV with down-regulated I_{K1} would have relatively depolarized resting potentials, which might lead to decreased velocity of conduction and favor reentry. Furthermore as hearts hypertrophy, the ratio of myocytes (conducting slowly at 0.4 M/s) to Purkinje fibers (conducting rapidly at 2-5 M/s) ([Opie, 2004](#)) increases, thus favoring still further the likelihood of reentry. Similarly, pro-arrhythmic cell-to-cell uncoupling has been previously observed in rabbits with dilated hearts ([Mills et al., 2008](#)). These reports are consistent with the higher incidence of TdP in hypertrophic animals, as well as with the prolonged QRS observed.

Clinical relevance

In the clinic it is generally accepted that the right ventricle is electrically more sensitive to mechanical stimuli/provocations from cardiac catheters than the left ventricle. Furthermore, it is thought that catastrophic ventricular fibrillation occurring when the anterior aspect of the human thorax is traumatized (commotio cordis) originates from the right ventricle ([Chen et al., 2004](#)). It is well-known that ectopic activity occurs more prevalently in patients with ventricular hypertrophy, and supraventricular arrhythmias occur commonly in patients with pulmonary disease ([Hanrahan et al., 2008](#); [Monserrat et al., 2003](#)), and patients with pulmonary disease frequently have right ventricular hypertrophy. Thus it is not surprising that rabbits with ventricular hypertrophy have

ventricular arrhythmias, albeit there is no data supporting the contention that the unique arrhythmia, torsades de pointes, occurs more frequently with right ventricular hypertrophy. The results of the current study confirm such clinical notions, highlighting the torsadogenic potential of right-ventricular remodeling.

Limitation(s)

Plasma concentrations of drugs were not measured, however drugs were given intravenously and doses were used that are recommended in the literature and that we have used previously and shown to be torsadogenic.

Echocardiography assessment of left ventricular and interventricular septal dimensions utilized standards developed in Veterinary Cardiology and specifically for rabbits ([Thomas et al, 1993](#); [Moura et al., 2009](#)), but the methods failed to interrogate right ventricular dimensions. However hypertrophy of all chambers was confirmed by postmortem measurements. Heart weight- body weight (HW: BW) ratios for all animals that were banded were greater than for animals sham-banded, but the HW: BW ratios did not differ among the groups with hypertrophy although it appeared to be greatest for rabbits with BVH (3.88 ± 0.14 for BVH versus 3.33 ± 0.07 for LVH and 3.26 ± 0.11 for RVH). Left ventricular and septal hypertrophy, but not right ventricular hypertrophy, was documented by echocardiography. The failure to observe RVH resulted from the point of interrogation by the echo probe.

Conclusion

This study shows that when compared to normal rabbits, animals with induced ventricular hypertrophy are more sensitive to the development of malignant arrhythmias in response to known torsadogens (sensitivity), while lacking increased arrhythmogenicity to known non-torsadogens (specificity). In agreement with such increased susceptibility to drug-induced TdP, rabbits with induced hypertrophy presented electrocardiographic signs of electrical remodeling suggestive of abnormal ventricular repolarization, developing prolonged QT-intervals and increased QT instability. Interestingly, and perhaps counter-intuitively, rabbits with induced right-ventricular hypertrophy were found to have the highest propensity/sensitivity to the development of drug-induced TdP, while normal controls and animals with left-ventricular hypertrophy showed the highest degree of resistance. In fact, the presence of a remodeled/hypertrophied left-ventricle in animals with concomitant right-ventricular hypertrophy (i.e., bi-ventricular) decreased the propensity to drug-induced TdP. When considered together, the results of this study strongly indicate that induced right-ventricular electrical remodeling plays a predominant role in the development of TdP, and therefore, models pursuing forms of right-ventricular disease may provide a more sensitive torsadogenic-screening paradigm than normal animals.

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