Rabbit Models of Cardiovascular Diseases for Preclinical Safety Pharmacological Studies

A. Kijtawornrat1,2*, N. Saengklab1, V. Limprasut1, P. Pirintr1, S. Kalandakanond-Thongsong1, K. Tachampa1, T. Kaewamatawong3, S. Sawangkoon3, C. Buranakarl1, N. Chaiyabut3
1Department of Physiology, Faculty of Veterinary Science
2Research study and testing of drug’s effect related to cardiovascular system in laboratory animal research clusters, Chulalongkorn University, Bangkok 10330, Thailand
3Department of Veterinary Bioscience and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand
4Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand
*Corresponding author: kanusak@hotmail.com

Keywords: dogs, heart diseases, preclinical studies, QT interval, rabbits, safety pharmacology

Introduction
Heart disease appears to be an independent risk factor for development of life-threatening arrhythmias such as torsades de pointes, ventricular premature complexes, ventricular tachycardia, and ventricular fibrillation (1, 2). Recently, acquired torsade de pointes was produced in dogs (3, 4) and rabbits (5) with long-standing complete AV block, whereas it can be produced only with rather dramatic alteration in plasmolytes (6) or with multiple drugs (7) in normal animals. Currently, most preclinical trials to test for torsadogenicity test only for surrogates of torsade de pointes, the most common surrogate being prolongation of QTc. Prolongation of QTc is a common effect of certain cardiovascular and non-cardiovascular drugs. In addition, not all drugs that prolong the QT interval are associated with an increased risk for torsade de pointes. In fact it is thought that the true mechanism for development of torsade de pointes is altered calcium kinetics resulting from calineurin-phosphorylation of ryanodine channels (8). Thus a model that develops torsade de pointes may well be superior for testing, directly, for a torsadogenic potential rather than merely testing for a surrogate, i.e., prolongation of QTc. This paper describes models of (1) left ventricular failure produced by coronary artery ligation in rabbits, (2) left ventricular hypertrophy produced by aortic banding in rabbits, (3) right ventricular hypertrophy produced by pulmonary arterial banding in rabbits, and (4) biventricular hypertrophy in rabbits. Those models, when exposed to torsadogenic compounds or other proarrhythmic drugs, develop torsade de pointes or other forms of ventricular arrhythmias.

Materials and Methods
Animal preparation and surgical procedures
New Zealand White rabbits were anesthetized with ketamine (35 mg/kg) and xylazine (5 mg/kg) administered intramuscularly. Animals received 100% oxygen (at the rate of 400-600 ml/min) and isoflurane (at the rate of 0.5-1.0) through a loose-fitting face mask designed for small animal. Surgery for LAD ligation in rabbit was described previously (9). In brief, rabbits were place in dorsal recumbency, and surgical anesthetia was confirmed by the absence of the pedal reflex. Both the left anterior descending and the major apical branch, from the left circumflex, coronary arteries were ligated at the midpoint between the starting point of the major branch and the cardiac apex using a 4/0 monofilament polypropylene suture. Muscle layers and skin were closed with simple interrupted sutures using 4/0 nylon and skin stapler, respectively. Post-operatively rabbits were given 0.03 mg/kg buprenorphine IM TID and 12 mg/kg enrofloxacin IM BID, for 3 days. Surgery for aortic banding and pulmonary artery banding were described previously (10-12). The surgical technique and pre/post-operative care are similar to the process of LAD ligation.

Echocardiographic assessment of left ventricular function
Echocardiographic examination was performed under light ketamine/xylazine sedation (15 mg/kg and 3 mg/kg respectively) on the day before coronary ligation and 4 weeks after surgery (rabbit with LAD ligation) or 3 months after surgery (rabbit with artery ligation). The rabbit was placed in right lateral recumbency with an area denuded to allow images to be obtained from the dependent right hemithorax. Echocardiographic recordings were made with simultaneous electrocardiogram (ECG), and all raw data was captured digitally for off-line measurement. Left ventricular function was assessed by measurement of left ventricle internal dimensions during systole and diastole, which allowed for calculation of a shortening fraction (SF). All Echocardiographic images were acquired and analyzed by a single experienced operator. Means±SEM was calculated for SF and was compared between baseline and 3 weeks after surgery with paired t test.

Experimental protocol
Animals were anesthetized. The right marginal ear vein was cannulated for IV administration of drug. The right and left thoracic limb electrodes are attached to the right and left hemithoraces, the electrocardiograph is switched to limb lead I, and a bipolar transthoracic ECG is obtained. Tracings were obtained continuously for a total of 150 min while the
rabbits were anesthetized. Each rabbit was given, intravenously over 10 minutes, 0, 5, 10, 20 and 40 µg/kg of dofetilide with 30 minutes between doses.

**Results and Discussion**

After surgery, ventricular remodeling developed continuously in those rabbits. First, structural remodeling was developed as ventricular dilation (LAD ligation) or ventricular hypertrophy (artery banding). Secondly, electrical remodeling was developed as alteration in QT, QTc, and short-term variability of QT interval. Finally, functional remodeling was also developed as reduced ejection fraction (EF) and shortening fraction (SF). Those models were also developed torsades de pointes arrhythmias when exposed to dofetilide. In conclusion, 1) disease models predict drug-induced toxicity in man; 2) models have high yield for production and are cost-effective and humane; 3) models not only manifest signals for being arrhythmic but actually develop arrhythmias; and 4) preclinical studies should investigate all CV parameters which if affected by a drug may translate to morbidity and/or mortality.

**Acknowledgements**
The authors would like to thank Ratchadapiseksomphot Endowment Fund (GSTAR 56-008-31-001), Chulalongkorn University, Bangkok, Thailand.

**References**