Torsade de Pointes

Risk prediction and role of ventricular activation

Thom R.G. Stams

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Torsade de Pointes:
Risk prediction and role of ventricular activation

Torsade de Pointes: risicopredictie en rol van ventriculaire activatie

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op maandag 16 juni des ochtends te 10.30 uur

door

Thom Reinier Gerardus Stams

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Preface

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Preface

Aims

A central aim in this thesis is the risk prediction of Torsade de Pointes (TdP) arrhythmias. But the ultimate goal is to evaluate the role of altered ventricular activation, especially whether a chronic dyssynchronous ventricular activation pattern can have a proarrhythmic effect.

The normal heart beat

Cardiac contraction is regulated by excitation contraction coupling.[1] Hence for effective pump function the heart requires an organized electrical depolarization of the tissue (excitation). Regular heart beats are initiated in the sino-atrial node. From that area, first electrical impulse conduction takes place to depolarize the atria. Due to the slowly conducting AV-node, atrial contraction can take place just before ventricular contraction, thereby improving ventricular filling (preload). The ventricles are depolarized via a specialized conduction system to cause a rapid, well-coordinated electrical activation. This conduction system consists of the common bundle of His, which branches into a left and right bundle branch and finally into Purkinje fibers, which are located subendocardially in the heart. The other cardiomyocytes are depolarized via cell-to-cell conduction.

The electrical activity of the heart can be visualized in detail with the electrocardiogram (ECG). An example of an ECG, recorded in an anesthetized dog, is shown in *Figure 1* (left side).

Electrical activity at the cellular level: the action potential

In isolated cells action potentials can be recorded (*Figure 2*). At rest, in between the heart beats, cardiomyocytes have a negative resting membrane potential. This is caused by differences in ion concentrations: the intracellular potassium concentration is high, whereas the intracellular sodium concentration is low, compared to the concentration in the extracellular space. These concentration differences are actively maintained by the cells using the Na^+/K^+ ATPase and are used as energy source for the generation of action potentials. At rest the conductance of potassium is larger than that of sodium ions resulting in a negative membrane potential, whereas upon a rise in membrane potential (due to depolarization of a neighboring cell or by artificial electrical stimulation), voltage-gated (selective) sodium channels rapidly open to cause a rapid depolarization. Inactivation of sodium channels and voltage-dependent activity of multiple other channels underlie the normal shape of the action potential.

Arrhythmias

Cardiac arrhythmias can be subdivided into two categories: supraventricular (atrial) arrhythmias

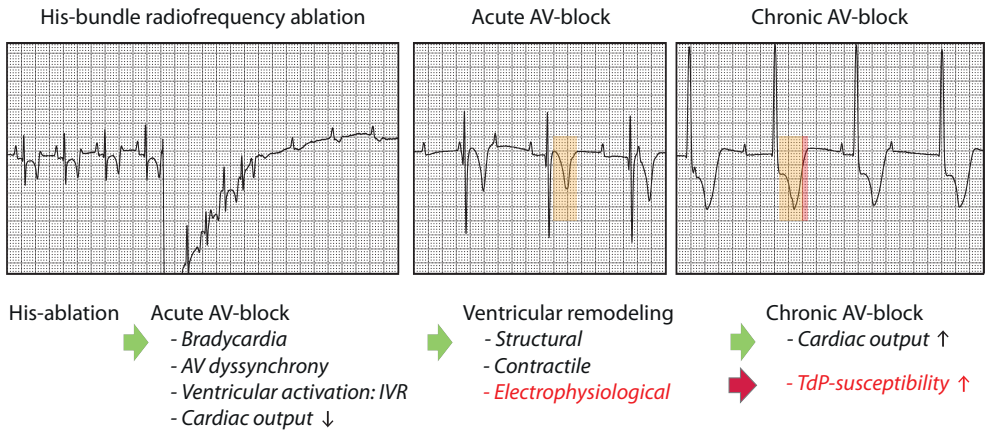

Figure 1. Brief overview of the canine chronic complete AV-block model

The panels show ECG recordings (lead II) from an individual dog. Under general anesthesia AV-block is created by radiofrequency His-bundle ablation (left panel), which results in emergence of spontaneous, slow idioventricular rhythm (middle panel). This results in an acute drop in cardiac output. The period during the experiment, immediately after induction of AV-block, is called acute AV-block. On the longer term the heart is able to restore its cardiac output almost completely, due to ventricular adaptations (remodeling). The ventricular remodeling, in particular the electrical remodeling, causes an increased susceptibility to Torsade de Pointes (TdP) arrhythmias at chronic (≥ 2 weeks) AV-block. In the example the QT prolongation is visualized in red (note that the focus of idioventricular rhythm in this dog is changed; but it is well known that on average QT prolongs considerably after chronic AV-block).

and ventricular arrhythmias. A relatively common arrhythmia is the supraventricular ventricular arrhythmia atrial fibrillation. This arrhythmia can reduce quality life (e.g. reduction of exercise tolerance) but also cause more severe symptoms (e.g. due to thromboembolic events) and the arrhythmia is associated with an increased risk of development of heart failure and increased mortality rate.[2]

One of the treatment options for atrial fibrillation is anti-arrhythmic drug treatment using class III anti-arrhythmic drugs that prolong repolarization. Disadvantage is that these drugs also have an effect on ventricular repolarization which can result in a serious, immediately life-threatening proarrhythmic adverse event: Torsade de Pointes (TdP).[2]

TdP is a polymorphic ventricular tachycardia which mostly terminates spontaneously but can also degenerate into ventricular fibrillation and cause sudden cardiac death. TdP was first described by Dessertenne, in 1966.[3] He coined the term 'Torsades de Pointes' because of the characteristic twisting of the QRS peaks around the isoelectric line, observed during longer lasting arrhythmias. TdP is typically associated with delayed repolarization, observed as QT prolongation on the surface ECG, although a short-coupled variant without QT prolongation exists as well.[4]

Mechanisms of Torsade de Pointes and risk factors

The induction and perpetuation of the arrhythmia can be explained by the following two mechanisms: non-reentry based focal activity and re-entry. The focal activity during initiation of TdP is usually ascribed to early (or delayed) afterdepolarizations, but especially the most important mechanism for perpetuation has been a frequent matter of debate.[5,6]

Many different drugs, both cardiovascular and non-cardiovascular, have been associated with TdP and sometimes even withdrawn from the market due to the risk of TdP. An example is cisapride, a gastrointestinal prokinetic drug used for treatment of gastroesophageal reflux disease. This drug was introduced on the market by Jansen Pharmaceutica in 1993 and withdrawn in 2000, after accumulation of many reports of ventricular tachycardia not related to other causes and sometimes resulting in sudden arrhythmic death.[7]

Both congenital and acquired risk factors for TdP exist.[8] Patients with a congenital long QT syndrome are at a highly increased risk, but the arrhythmia is also seen in the general population, often in the setting of multiple risk factors and often linked to a drug that prolongs the QT interval as side effect. However, many other risk factors exist, including AV-block, bradycardia and chronic heart failure.[8] This is summarized in *Figure 3*.

QT prolongation is often used to predict the risk of TdP, but the parameter is not closely associated with the arrhythmic outcome (this is also discussed in Chapter 3). For example, prolongation of repolarization can be anti-arrhythmic against TdP.[9,10] Thus, other factors are also important for proarrhythmic risk, e.g. whether the drug could enhance formation of early afterdepolarizations and spatial dispersion of repolarization.[5]

To improve risk prediction, in this thesis we also evaluate the use of other parameters that have been associated with the TdP outcome: especially beat-to-beat variability of repolarization, but also other parameters including spatial dispersion (the difference between left and right ventricular monophasic action potential duration; ΔMAPD) and the interval from T wave peak to end ($T_{peak\text{-}end}$).[11-14]

The CAVB dog model

The anesthetized, chronic, complete AV-block (CAVB) dog model is characterized by a highly enhanced susceptibility to drug-induced Torsade de Pointes, related to so-called ventricular remodeling which occurs as a consequence of the AV-block creation (*Figure 1*). The model has been described in detail by Oros, *et al.*[11] The ventricular remodeling, which includes structural, electrical and mechanical adaptations of the heart, results in an almost complete restoration of cardiac output. The biventricular hypertrophy (right ventricle > left ventricle) is developing most quickly at one week, but takes about six to eight weeks to reach is plateau. The increased TdP susceptibility has been demonstrated as early as two weeks after creation of AV-block and remains stable over time during multiple months. The class III antiarrhythmic drug dofetilide, a specific blocker of the rapidly activating delayed rectifier potassium current (I_{Kr}), is nowadays used as reference compound to test TdP susceptibility in the model. The increased susceptibility

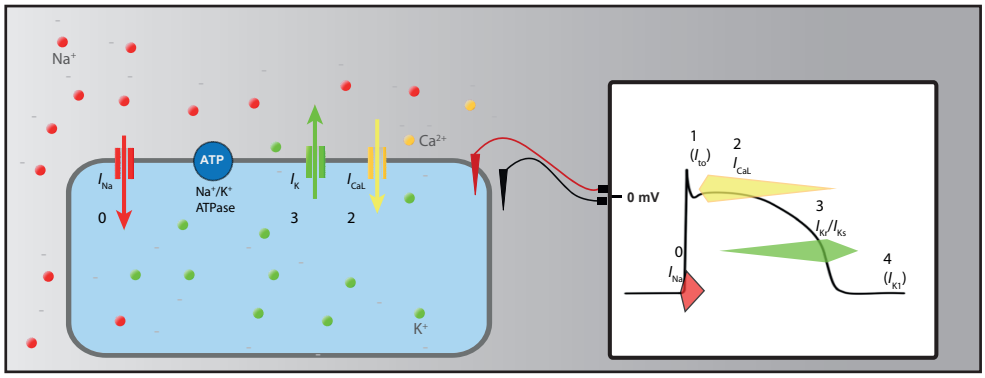

Figure 2. Cardiac action potential

A highly simplistic overview of the phases of the cardiac ventricular action potential (right panel) and the most important transmembrane ionic currents involved. The left panel represents a single cardiomyocyte (blue).

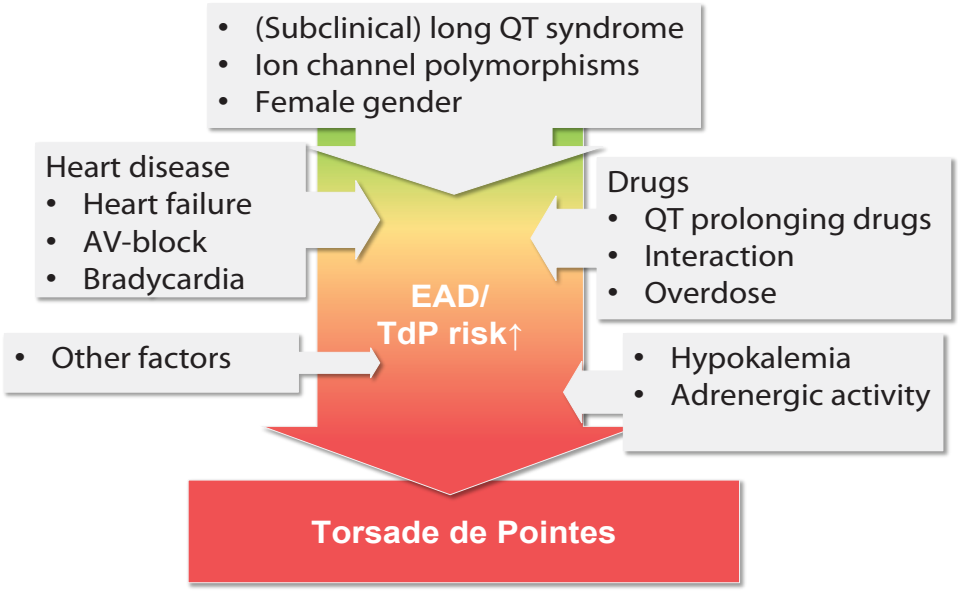

Figure 3. Schematic overview of congenital and acquired risk factors for Torsade de Pointes

Based on the review of Roden.[8] EAD, early afterdepolarization.

to TdP is closely related to the QT prolongation, which is also present at two weeks and has then (almost) reached its plateau as well.[15-19]

Arrhythmias in the CAVB model have been linked to both early and delayed afterdepolarizations in vitro, but also in vivo using monophasic action potential (MAP) recordings.[16,20,21] In isolated cardiomyocytes, early afterdepolarizations can be induced with class III anti-arrhythmic drugs, while they can be suppressed with anti-arrhythmic drugs.[22,23] In the CAVB model focal activity, induced by afterdepolarizations, seems to be important for initiation and perpetuation, although re-entry seems to play a role as well.[24]

The CAVB model is primarily developed to test the safety of drugs (cardiac safety pharmacology), especially if some concerns have arisen, e.g. due to observation of QT prolongation in model systems or patients.[25-29] The model is also used to evaluate anti-arrhythmic strategies against TdP.[22,23,30]

Dyssynchronous ventricular activation and cardiac resynchronization therapy (CRT)

Originally the proarrhythmic remodeling in the CAVB dog model has been ascribed to the bradycardia-induced volume overload, but the abnormal ventricular activation also contributes: in a study conducted by Winckels, *et al.*[31] bradycardia was induced by AV-block and pacing at lowest captured rate, while a 'physiological' activation pattern was maintained by high-septal pacing. These dogs showed attenuated TdP-severity compared with the control group of AV-block dogs with chronic, unpaced idioventricular rhythm.

Dyssynchronous ventricular activation (DVA) by left bundle branch block or right ventricular pacing can impair the mechanical function of the heart and result in detrimental ventricular remodeling.[32] The dyssynchrony of activation can be reversed with cardiac resynchronization therapy (CRT), which has been shown in dogs with isolated left bundle branch block, created by radiofrequency ablation.[33,34] CRT is currently an important treatment for patients with heart failure and left bundle branch block.[35] In this thesis we further and more specifically explore the effects of dyssynchronous activation and CRT on arrhythmogenesis of TdP, and the risk prediction with electrophysiological parameters.

Thesis outline

Atrial fibrillation is a much less severe arrhythmia than ventricular tachycardias, like TdP, but this arrhythmia is very common, especially at older age. Current drugs are not always effective and/or have serious side effects, including drug-induced TdP. As a consequence there is a huge potential market for novel drugs and therefore a lot of research is aimed at treatment of this disease. In *Chapter 2* we study the safety of the novel drug K201 (or JTV-519; developed by Japan Tobacco in the eighties) in the CAVB dog model. The safety evaluation is based on the induction of ventricular arrhythmias but we also used electrophysiological surrogate parameters, including QT interval and beat-to-beat variability of repolarization duration. Because K201 is

a multi-channel blocker that acts primarily by stabilizing the ryanodine receptor, this study was also interesting from a mechanistic point of view. *Chapter 3* is an invited commentary, accompanying a study by Milberg, *et al.*[36] in which the safety and anti-arrhythmic effects of the calcium antagonist verapamil were studied in a rabbit model of heart failure. In *Chapter 4* we evaluate the relevance of the electromechanical window (EMW; the interval from the end of QT to end of mechanical relaxation) in risk prediction of TdP, based on an analysis of previous experiments that had been performed in the dogs with complete AV-block. In *Chapter 5* we evaluate the safety (anti- and proarrhythmic effects) of DVA combined with bradycardia, induced by chronic pacing from the right ventricular apex at lowest captured rate, in dogs with AV-block. This site is currently still the standard site for ventricular pacing, in spite of the fact that this location is mechanically not optimal and results in heart failure in a minority of patients.[32] In *Chapter 6*, we include these results in a retrospective analysis, in which arrhythmia score and the initial rise of monophasic action potential duration after dofetilide (quantified as time till 25-ms increase, T25) are introduced as novel parameters to improve quantification of TdP-severity and the risk of TdP, respectively. In *Chapter 7* we explore the relevance of preload variability in the mechanism of beat-to-beat variability of repolarization duration in the CAVB model. In *Chapter 8* the proarrhythmic effect of chronic DVA without bradycardia is evaluated. In addition, the reversibility with chronic cardiac resynchronization therapy (CRT) is studied. The novel parameters from *Chapter 6* are also applied in this study. The final chapter, *Chapter 9*, is a general discussion.

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Chapter 2

Effects of K201 on repolarization and arrhythmogenesis in anesthetized chronic atrioventricular block dogs susceptible to dofetilide-induced Torsade de Pointes

Thom R.G. Stams[1,a], Avram Oros[1,a], Roel van der Nagel[1], Jet D.M. Beekman[1], Paul Chamberlin[2], Howard C. Dittrich[2], Marc A. Vos[1]

[1] *Department of Medical Physiology, Division Heart & Lungs, University Medical Center Utrecht, Utrecht, The Netherlands*

[2] *Sequel Pharmaceuticals, San Diego, United States of America*

[a] *Both authors contributed equally as first authors*

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ABSTRACT

The novel antiarrhythmic drug K201 (4-[3-{1-(4-benzyl)piperidinyl}propionyl]-7-methoxy-2,3,4,5-tetrahydro-1,4-benzothiazepine monohydrochloride) is currently in development for treatment of atrial fibrillation. K201 not only controls intracellular calcium release by the ryanodine receptors, but also possesses a ventricular action that might predispose to Torsade de Pointes arrhythmias. The anti- and proarrhythmic effects of K201 were investigated in the anesthetized canine chronic atrioventricular block model. Two doses of K201 (0.1 and 0.3 mg/kg/2min followed by 0.01 and 0.03 mg/kg/30min i.v.) were tested in four serial experiments in dogs with normally conducted sinus rhythm (n=10) and in Torsade de Pointes-susceptible dogs with chronic atrioventricular block. Susceptibility was assessed with dofetilide (0.025 mg/kg/5min i.v.). Beat-to-beat variability of repolarization was quantified as short-term variability of left ventricular monophasic action potential duration. In dogs with normally conducted sinus rhythm, both doses of K201 prolonged ventricular repolarization whereas only the higher dose prolonged atrial repolarization. At chronic atrioventricular block, dofetilide induced torsade de pointes in nine of ten dogs. K201 did neither suppress nor prevent dofetilide-induced torsade de pointes. K201 dose-dependently prolonged ventricular repolarization. In contrary to the lower dose, the higher dose did increase beat-to-beat variability of repolarization (from 1.2±0.3 to 2.9±0.8 ms, $P<0.05$) and resulted in spontaneous, repetitive torsade de pointes arrhythmias in one of seven dogs; Programmed electrical stimulation resulted in torsade de pointes in two more dogs. In conclusion, both doses of K201 showed a class III effect. No relevant antiarrhythmic effects against dofetilide-induced Torsade de Pointes were seen. Only at the higher dose a proarrhythmic signal was observed.

Keywords: K201; JTV519; Beat-to-beat variability of repolarization; ventricular arrhythmia; cardiac electrophysiology; safety pharmacology

INTRODUCTION

The novel drug K201 (or JTV519), a 1,4-benzothiazepine derivative, is known to have anti-arrhythmic and cardio-protective properties[1] against intracellular calcium overload,[2] ischemia-reperfusion injury,[3,4] heart failure,[5] catecholaminergic polymorphic ventricular tachycardia and sudden cardiac death.[6] These effects have been explained by the ability of K201 to suppress (diastolic) intracellular Ca^{2+} leak from the cardiac ryanodine receptor (ryanodine receptor 2) by stabilization of peptidyl-prolyl cis-trans isomerase FK506 binding protein 1B, 12.6 kDa (FKBP1B, also known as calstabin2) binding.[6,7] Still, the precise mechanisms of action are controversial.[1,8] Initially, low dosages of K201 (8 μg/kg/min) were studied in various animal models (catecholaminergic polymorphic ventricular tachycardia mice, guinea pigs, dogs with pacing induced heart failure). At higher doses (30 μg/kg/min), K201 suppressed atrial fibrillation experimentally [9-11] and currently K201 is in development for clinical treatment of atrial fibrillation.

Besides preventing leakage of calcium from ryanodine receptor 2, K201 is known to block numerous ion channels, resulting in prolongation of action potential duration. This includes block of the muscarinic acetylcholine receptor-operated K^+ current $I_{K,Ach}$ (IC_{50}=0.12 μM), the rapidly activating and deactivating delayed rectifier K^+ current (I_{Kr}) in atrial (IC_{50} = 0.41 μM)[9] and ventricular (IC_{50} 1.2 μM)[12] cardiomyocytes, the sodium current I_{Na} (IC_{50}=1.2-2 μM), the L-type calcium current I_{CaL} (IC_{50}=3 μM), the inward rectifier potassium current I_{K1} (IC_{50}=5 μM)[12] and $alpha_1$-adrenergic receptors[2] and affects $I_{K,ATP}$-opening properties.[4]

It is well known that drugs specifically blocking I_{Kr} prolong the QT interval and can cause Torsade de Pointes arrhythmias as an adverse effect. With K201, atrial action potential duration lengthening[9] and ventricular action potential duration shortening[13] have been described in guinea pigs. In contrast, other studies in rabbits and dogs reported QT prolongation[10,14,15] without occurrence of Torsade de Pointes (10-400 μg/kg/min). In the methoxamine-sensitized rabbit, proarrhythmic activity depends on alpha-adrenergic stimulation, which is a target of K201.[2,14] Thus it is possible that the anti-arrhythmic effect is based on this secondary pharmacology of K201. To best of our knowledge, the tendency of this drug to produce or prevent Torsade de Pointes in other animal models with no or less reliance on alpha-adrenergic stimulation has not been investigated. Thus, in present study the anti- and pro-arrhythmic effects of K201 were investigated.

In serial experiments, K201 was given at two doses to anesthetized dogs with either normally conducted sinus rhythm or remodeled hearts due to chronic, complete atrioventricular block. The analysis included measurements of beat-to-beat variability of repolarization, a parameter suggested to have more predictive power for the detection of pro-arrhythmic signals than the clinically most used parameter QT-interval.[16,17]

MATERIALS AND METHODS

All experiments were performed in accordance to the "European Directive for the Protection of Vertebrate Animals used for Experimental and Scientific Purpose, European Community Direc-

tive 86/609/CEE" and with approval from "The Committee for Experiments on Animals" of Utrecht University, The Netherlands.

Anesthesia and general experimental protocol

Ten adult purpose-bred mongrel dogs (Marshall, USA; body weight: 23±2 kg, 4 females) were included. Experiments were performed under general anesthesia after overnight fasting. Premedication consisted of 0.5 mg/kg methadone, 0.5 mg/kg acepromazine and 0.02 mg/kg atropine i.m. Anesthesia was induced with pentobarbital (Nembutal 25 mg/kg i.v.) and maintained by isoflurane (1.5%) in a mixture of O_2 and N_2O (1:2). Appropriate care was taken during and after the experiments including use of a thermal mattress to maintain body temperature, saline administration to prevent volume depletion (0.5 L 0.9% NaCl i.v.), and administration of antibiotics (ampicillin 1000 mg i.v. and i.m., before and after the experiment, respectively; KELA NV, Belgium) and analgesics (buprenorphine 0.015 mg/kg i.m.; AST Pharma BV, Netherlands). In between experiments, at least two weeks expired to allow full recovery of the animals.

A standard 6-lead surface ECG and four precordial leads were recorded throughout the experiments. Endocardial monophasic action potentials were recorded from the left and right ventricular wall (catheters: Hugo Sachs Electronics, Germany). In experiments during sinus rhythm and chronic, complete atrioventricular block the latter catheter was temporarily repositioned to record signals from the right atrium as well.

K201 (4-[3-{1-(4-benzyl)piperidinyl}propionyl]-7-methoxy-2,3,4,5-tetrahydro-1,4-benzothiazepine monohydrochloride; $C_{25}H_{32}N_2O_2S{\cdot}HCl$; molecular weight 461.07; Sequel Pharmaceuticals Inc., San Diego, CA, USA) was provided in a concentration of 2 mg/ml. The two doses studied were 0.1 and 0.3 mg/kg/2min i.v. followed by maintenance infusion of 0.01 or 0.03 mg/kg/min respectively for 30 min (in the text and figures shortened to K201 lower (dose) and K201 higher (dose), respectively). Blood samples were taken at regular time points and sent to Sequel Pharmaceuticals Inc. for analysis of the plasma concentrations.

Experimental protocol

The four serial experiments are illustrated in *figure 1*: in experiment 1, ventricular and atrial repolarization parameters were determined before and after the lower (n=5) or higher (n=5) dose of K201 in dogs with normally conducted sinus rhythm (unremodeled heart). Only after the higher dose, left ventricular pressure was measured using a 6F pressure catheter (Sentron, Roden, Netherlands). At the end of this experiment, irreversible third-degree atrioventricular block was induced by radiofrequency His-bundle ablation as previously described.[18]

In experiment 2, at chronic, complete atrioventricular block due to at least three weeks remodeling of the heart,[19] the dogs were challenged with the specific I_{Kr} blocker dofetilide (0.025 mg/kg in 5 min i.v.) to determine the inducibility of Torsade de Pointes (*Figure 1*, experiment 2). Dofetilide infusion was stopped when Torsade de Pointes occurred and the exact administered

dose was recorded. In inducible dogs, the anti-arrhythmic effects of the two doses of K201 were investigated.

In the last two experiments (*Figure 1*, experiment 3a/3b) electrophysiological effects of both doses of K201 were studied in a random cross-over design. In addition, the anti-arrhythmic potential of K201 to prevent dofetilide-induced Torsade de Pointes was assessed by re-administering the arrhythmogenic dose of dofetilide. Besides the regular electrophysiological parameters, right ventricular and right atrial effective refractory period were determined at baseline and after K201 administration using programmed electrical stimulation (PES; *Figure 1*, hatched bars). Before measurements, steady state pacing was performed for 2 min. Right ventricular effective refractory period was determined by pacing from the right ventricular monophasic action potential catheter using a train of eight paced beats with 800 ms cycle length and a pacing output of two times the diastolic threshold, followed by an extra stimulus using a decremental design (starting from a cycle length of 300 ms) in steps of 5 ms till the effective refractory period was reached. Right atrial effective refractory period was determined by pacing from the right atrial monophasic action potential catheter at 325 ms drive cycle length with a pacing output four times the diastolic threshold.

Data analysis

RR and QT interval in lead II, left and right ventricular monophasic action potential duration at 90% repolarization and right atrial monophasic action potential duration at 50% repolarization were measured at various time points, off-line and semi-automatically, using a computer program (ECG-Auto, EMKA Technologies, France). QT intervals were corrected for heart rate (QTc) with Van de Water's method.[20] Interventricular dispersion of repolarization was calculated as the difference between the left and right ventricular monophasic action potential duration. Data measurements were averaged from five consecutive beats. Beat-to-beat variability of repolarization duration was quantified as short-term variability from left ventricular monophasic action potential duration at 90% repolarization (STV_{LV}) of 30 consecutive beats: $STV_{LV} = \sum|X_n - X_{n-1}| / (30\sqrt{2})$, where X represents left ventricular monophasic action potential duration.[21] Short-term variability measurements were performed at fixed time points or, in case of proarrhythmia, at the last possible preceding interval consisting of at least 30 consecutive beats. Left ventricular blood pressure recordings were analyzed using custom made software (ECGView, Maastricht University, Netherlands) to estimate the maximum rate of rise of left ventricular pressure (dP/dt max).

Quantification of arrhythmias

Ectopic beats were defined as beats initiating before the end of the preceding T wave. Distinction between single or multiple (two to four) ectopic beats was made, as the latter are considered more pro-arrhythmic.[22] Torsade de Pointes was defined as a polymorphic ventricular tachyarrhythmia of at least five beats and with a twisting shape (variable axis). A dog was considered inducible when Torsade de Pointes occurred at least three times during the 10 min interval after

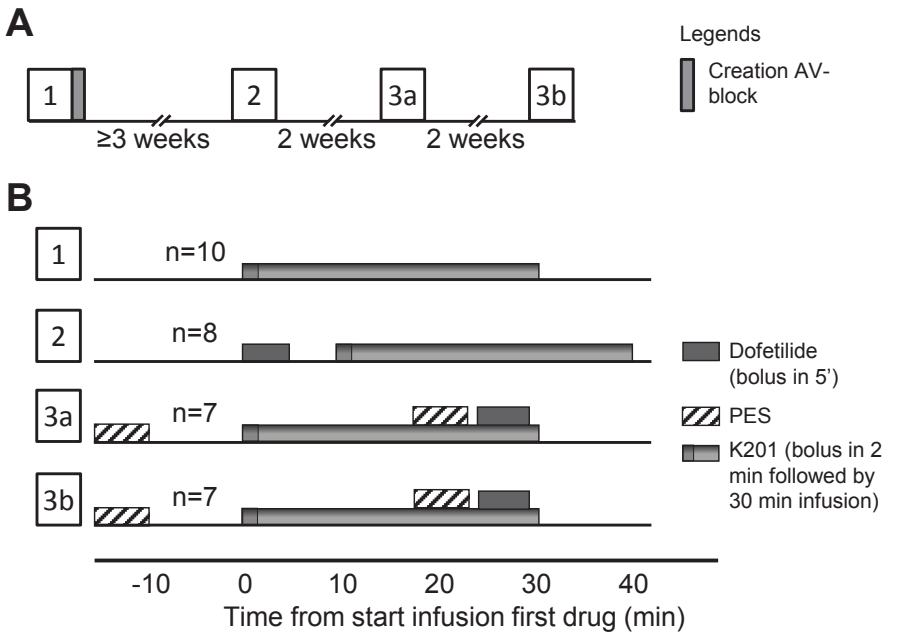

Figure 1: Overview of the serial experiments performed in this study

Each dog was planned to undergo four experiments under general anesthesia (A). Details of the experiments (B): in experiment 1, prior to induction of atrioventricular block, the ventricular and atrial electrophysiological effects of the lower (n=5) and the higher doses (n=5) of K201 were tested in normal hearts. In experiment 2, at least three weeks later (chronic atrioventricular block) susceptibility to dofetilide-induced Torsade de Pointes was assessed (n=10). In inducible dogs, K201 was given in the second part of the experiment to study the anti-arrhythmic potential of the higher (n=3) or lower dose (n=5). In experiments 3a/3b the electrophysiological and pro-arrhythmic properties of the lower and higher doses of K201 were evaluated in a random crossover design (n=7). In the second part of these experiments dofetilide was administered to test the antiarrhythmic (preventive) potential of K201.

start of dofetilide infusion. If Torsade de Pointes did not stop within 10 s or if the arrhythmia degenerated into ventricular fibrillation, electrical cardioversion was performed via thoracic patches placed in advance. The number and duration of Torsade de Pointes arrhythmias were quantified over the 10 min period after the start of dofetilide administration and compared to 10 min intervals at baseline and after K201 administration (the 10 min interval after complete infusion of the bolus in experiment 2 or prior to programmed electrical stimulation in experiments 3a/3b; *Figure 1B*).

Statistical analysis

Data are expressed as mean ± standard deviation (SD). Comparisons of serial data were performed with one-way repeated measures analysis of variance (ANOVA) with post hoc Bonferroni correction (*Tables 1 and 3*), or a paired t-test (*Tables 2 and 4*). For non-parametric comparisons,

the Kruskal-Wallis test followed by Dunn's test or Wilcoxon signed-ranks test were used, respectively. Two-way repeated measures ANOVA followed by Bonferroni correction was used to study the dose and time dependent effects of K201 (*Figure 3*). McNemar's test was used for analysis of Torsade de Pointes inducibility. Used software was SigmaStat (Version 3.11, Systat Software Inc.). Statistical significance was defined as P≤0.05.

RESULTS

Because the plasma concentrations did not differ between sinus rhythm and chronic atrioventricular block dogs, we present them together (*Figure 2A*). Steady state plasma concentrations between 10 and 30 min after the start of K201 were roughly 300 (n=8) and 800 ng/ml (n=9), respectively. Immediately after the bolus (relevant for experiment 2), the plasma concentrations were higher.

Electrophysiological effects of K201 in anesthetized dogs with normally conducted sinus rhythm (experiment 1)

In general, K201 prolonged ventricular repolarization and slowed the heart rate, independent of dose (*Table 1*). This effect started to appear at 15 min and was clearly present at 30 min and was maintained after the infusion was stopped, till at least 45 min after start of infusion. The relative increase of QTc over time is shown in more detail in *figure 2B*. Only at the higher dose, K201 prolonged atrial repolarization (right atrial monophasic action potential duration at 50% repolarization). The higher dose was free of negative inotropic effects: left ventricular dP/dt max was not changed (from1340±193 mmHg/s in control to 1333±299 mmHg/s at 30 min, P=NS).

Suppressive effects of K201 on dofetilide-induced torsade de pointes (experiment 2)

In dogs with chronic atrioventricular block, dofetilide administration (n=10) resulted in a significant increase in most repolarization parameters, including QTc (from 435±68 to 550±96 ms, P<0.05) and short-term variability of left ventricular monophasic action potential duration (from 1.8±1.2 to 3.5±0.8 ms, P<0.05). Moreover, dofetilide caused reproducible Torsade de Pointes arrhythmias in nine out of ten canines. Arrhythmia quantification in this group (n=9) revealed 11±8 episodes of Torsade de Pointes, 5±4 defibrillations, 19±25 runs of multiple ectopic beats and 48±55 single ectopic beats during the 10 min observation period (vs. 0±0, 0±0, 0±1 and 12±37 at baseline, respectively; all P<0.05, except for the number of single ectopic beats (P=0.074)). Of the nine torsade de pointes-susceptible dogs, one animal did not receive K201 in the second part of the experiment, because a defect in the defibrillation patches necessitated immediate anti-arrhythmic intervention. For that purpose, we used the fast-acting, established antidote, levcromakalim.[17] In the remaining eight individuals, five dogs received the higher and three the lower dose of K201. The arrhythmogenic outcome measures of both dosages are pooled because the data were similar. K201 was not able to suppress dofetilide-induced Torsade de

Table 1. Electrophysiological effects of two doses of K201 in anesthetized dogs with normally conducted sinus rhythm (experiment 1)

	Baseline	15 min	30 min	45 min
K201 lower (n=5)				
RR	581 ± 37	598 ± 38	605 ± 35*	614 ± 42*
QT	268 ± 16	280 ± 17*	289 ± 17*	293 ± 17*
QTc	304 ± 16	315 ± 16*	323 ± 17*	327 ± 15*
LV $MAPD_{90}$	213 ± 19	224 ± 10	240 ± 17*	234 ± 20
RV $MAPD_{90}$	197 ± 5	211 ± 11*	210 ± 10*	-
ΔMAPD	16 ± 15	13 ± 12	29 ± 14	-
RA $MAPD_{50}$	82 ± 30	84 ± 16	83 ± 20	-
K201 higher (n=5)				
RR	589 ± 16	625 ± 30*	618 ± 42*	630 ± 51*
QT	264 ± 8	287 ± 11*	289 ± 10*	290 ± 13*
QTc	300 ± 8	319 ± 9*	323 ± 9*	322 ± 11*
LV $MAPD_{90}$	209 ± 21	226 ± 24	240 ± 20*	247 ± 11*
RV $MAPD_{90}$	190 ± 15	220 ± 9*	218 ± 16*	224 ± 18*
ΔMAPD	19 ± 32	6 ± 18	22 ± 16	23 ± 13
RA $MAPD_{50}$	101 ± 11	106 ± 1	125 ± 13*	-

LV, left ventricle; RV, right ventricle; MAPD90, monophasic action potential duration at 90% repolarization; ΔMAPD, difference between LV and RV MAPD90; RA MAPD50, right atrial monophasic action potential duration at 50% repolarization. Times represent minutes after start of infusion of K201. Two groups of dogs were analyzed with either the lower or the higher dose of K201.

*, P<0.05 vs. baseline.

Pointes. Moreover the severity of the arrhythmias was unchanged: neither the number of defibrillations (from 5±3 to 5±4, P=NS) nor the number of Torsade de Pointes episodes (from 11±8 to 15±11, P=NS) were reduced. In contrary, a trend towards reduction of the mean duration of the Torsade de Pointes episodes was observed after addition of K201 (from 10±7 s after dofetilide to 6±1 s after addition of K201, P=0.055). These effects of K201 on Torsade de Pointes were accompanied by a trend towards an increase in both the number of multiple ectopic beats (from 0±1 to 20±26 and 44±21, at baseline, after dofetilide and after addition of K201, respectively; post hoc test P<0.05 only for K201 vs. baseline) and the number of single ectopic beats (14±39, 52±58 and 139±96, at baseline, after dofetilide and after addition of K201 respectively; post hoc test P<0.05 only for K201 vs. baseline).

Electrophysiological effects of K201 in anesthetized dogs with chronic atrioventricular block

One dog was lost at the end of experiment 2, leaving eight dogs for the remainder of the protocol

Table 2. Electrophysiological effects of two doses of K201 in anesthetized dogs with chronic complete AV-block (experiments 3a/3b)

	Baseline	K201 18 min
K201 lower (n=7)		
RR	1144 ± 160	1206 ± 158*
QT	413 ± 54	487 ± 58*
QTc	400 ± 50	467 ± 55*
LV $MAPD_{90}$	309 ± 44	387 ± 62*
RV $MAPD_{90}$	273 ± 25	313 ± 28*
ΔMAPD	36 ± 29	74 ± 49*
STV_{LV}	1.0 ± 0.5	1.3 ± 0.7
RV ERP (n=6)	247 ± 25	253 ± 14
RA ERP (n=6)	123 ± 5	154 ± 17*
K201 higher (n=7)		
RR	1218 ± 172	1371 ± 215*
QT	429 ± 63	525 ± 67*
QTc	410 ± 61	493 ± 53*
LV $MAPD_{90}$	338 ± 51	458 ± 78*
RV $MAPD_{90}$	290 ± 22	360 ± 54*
ΔMAPD	48 ± 40	98 ± 61*
STV_{LV}	1.2 ± 0.3	2.9 ± 0.8*
RV ERP (n=4)	248 ± 26	293 ± 28*
RA ERP (n=3)	109 ± 17	142 ± 19

LV, left ventricular; RV, right ventricular; $MAPD_{90}$, monophasic action potential duration at 90% repolarization; ΔMAPD, difference between LV and RV $MAPD_{90}$; RA $MAPD_{50}$, right atrial monophasic action potential duration at 50% repolarization. STV_{LV}, LV $MAPD_{90}$ short-term variability; ERP, effective refractory period; RA, right atrial.

*, P<0.05 vs. baseline.

(experiments 3a/b). In experiments 3a and 3b one other dog was excluded due to occurrence of spontaneous Torsade de Pointes at baseline, leaving seven animals for the serial tests with K201.

K201 lower dose: 0.1 mg/kg/2min + 0.01 mg/kg/min for 30 min

The lower dose of K201 caused lengthening of most repolarization parameters, with the exception of right ventricular effective refractory period and short-term variability of left ventricular monophasic action potential duration (*Table 2*, upper part and *figure 3A*). No spontaneous Torsade de Pointes arrhythmias were seen with this dose. Only some single and multiple ectopic beats were seen; the latter in only two dogs (*Table 3*, upper part). Programmed electrical stimulation after administration of K201 did not induce Torsade de Pointes.

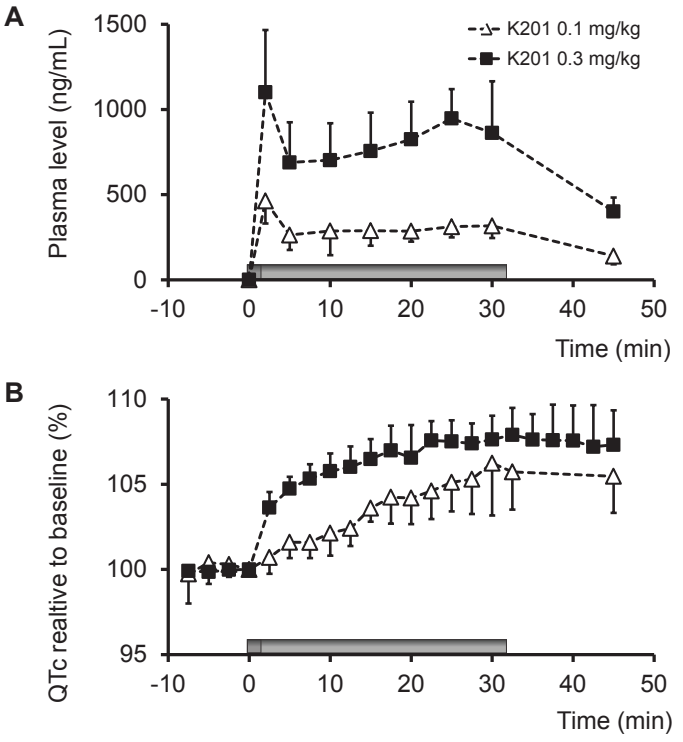

Figure 2. Plasma concentrations of K201 with its effect on QTc

A: Time-dependent plasma concentrations of K201 after bolus (2 min) and maintenance infusion (30 min) of the lower and higher dose of K201. The bar above the x-axis represents this infusion scheme. Please note that the maintenance infusion prevented rapid decline in K201 concentrations thereby creating a steady state.

B: Time-dependent effects of the lower (n=5) and higher (n=5) dose on QTc, relative to baseline at t=0 (start bolus infusion), in anesthetized dogs with normally conducted sinus rhythm.

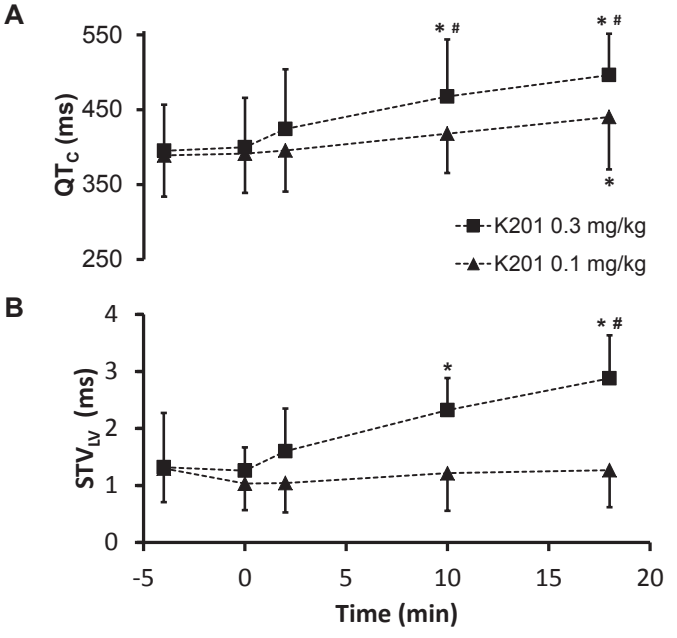

Figure 3. Dose-dependent electrophysiological effects of two dosages K201 on QTc and short-term variability of left ventricular monophasic action potential duration in dogs with chronic atrioventricular block

The higher dose increased both QTc (A) and short-term variability (B), whereas the lower dose only increased QTc. These effects were time-dependent.

STV_{LV}, short-term variability of left ventricular monophasic action potential duration; *, P<0.05 vs. baseline at t=0 min; #, P<0.05 higher vs. lower dose of K201.

Table 3. Pro-arrhythmic effects of K201 in anesthetized CAVB dogs (experiments 3a/b)

	Baseline 0-10 min	K201 0-10 min	K201 10-20 min
K201 lower (n=7)			
Inducibility, % (95%-CI)	0 (0-32)	0 (0-32)	0 (0-32)
TdP, n	0	0	0
T/TdP, s	No TdP	No TdP	No TdP
Defibrillations, n	No TdP	No TdP	No TdP
MEB, n	0	0.1 ± 0.4	0.7 ± 1.9
SEB, n	1.3 ± 1.9	2.4 ± 2.5	3.4 ± 8.6
K201 higher (n=7)			
Inducibility, % (95%-CI)	0 (0-32)	0 (0-32)	14 (1-53)
TdP, n	0	0	1.4 ± 3.4
T/TdP, s	0	0	2.3 ± 0.3
Defibrillations, n	No TdP	No TdP	0
MEB, n	0	1.1 ± 2.3	11 ± 24
SEB, n	0.3 ± 0.8	10 ± 18	40 ± 48

95%-CI, 95% confidence interval, modified Wald method; TdP, Torsade de Pointes; T/TdP, mean duration of TdP episodes; MEB, runs of multiple ectopic beats; SEB, single ectopic beats; No TdP, not calculable because no TdP arrhythmias were present; This table shows only spontaneous arrhythmias (i.e. occurring in the intervals before programmed electrical stimulation was performed).

K201 higher dose: 0.3 mg/kg/2min + 0.03 mg/kg/min for 30 min

The higher dose also slowed the heart rate and caused lengthening of all repolarization parameters (*Table 2*, lower part). QTc and short-term variability of left ventricular monophasic action potential duration were both increased dose-dependently (*Figure 3*). Between 10 and 20 min after the start of the higher dose of K201 (second observation period), short-term variability of left ventricular monophasic action potential duration significantly increased and nine self-terminating Torsade de Pointes arrhythmias appeared in one animal (*Figure 4*) with an average duration of 2.5±1 s. In addition, programmed electrical stimulation resulted in the induction of Torsade de Pointes in two additional animals, precluding effective refractory period measurements.

Anti-arrhythmic action of K201 in preventing dofetilide-induced torsade de pointes

The lower dose of K201 showed no preventive action: dofetilide was still able to initiate spontaneous Torsade de Pointes in five of six dogs (*Table 4*, upper part).

At the higher dose of K201, following programmed electrical stimulation, Torsade de Pointes reappeared and hindered further investigations. Therefore, only in three of seven dogs the preventive protocol of the higher dose K201 against dofetilide-induced pro-arrhythmia could be

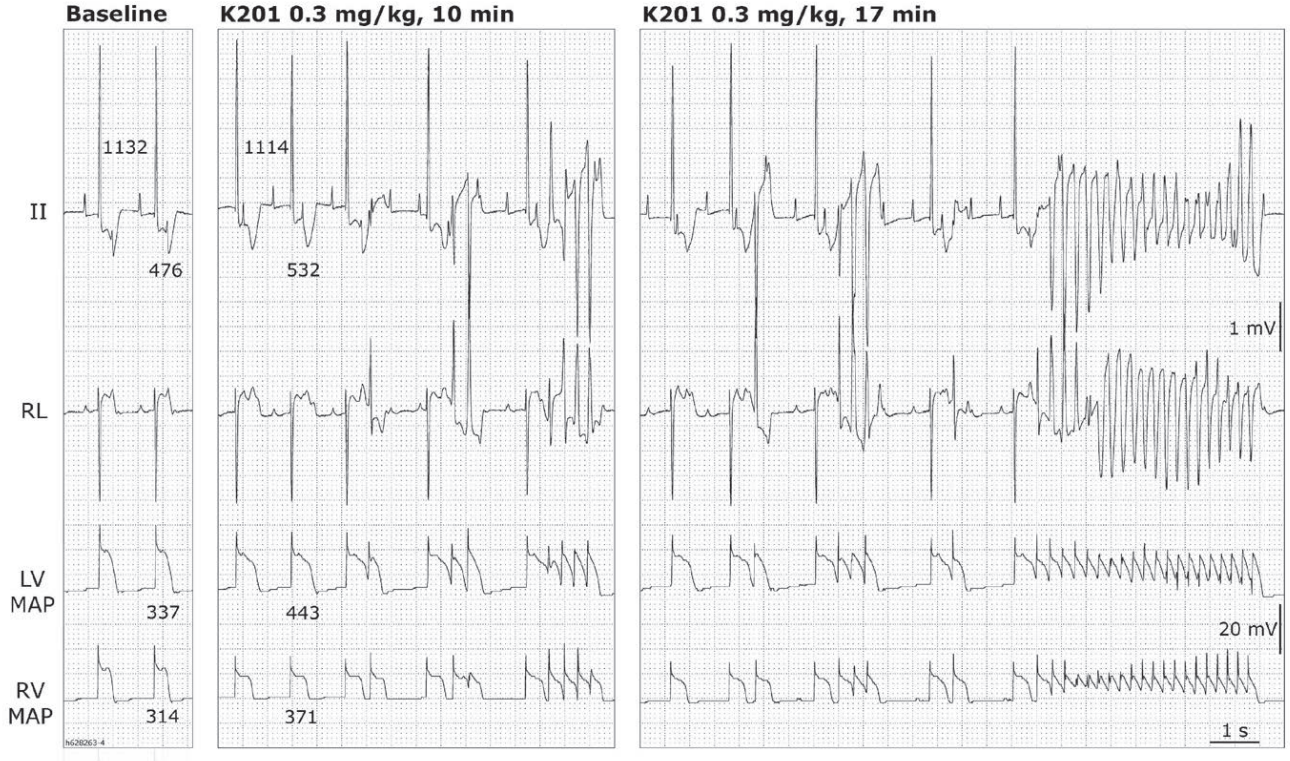

Figure 4: Individual example of torsade de pointes induction with the higher dose of K201

Two ECG leads (II and the precordial lead RL) and two endocardial monophasic action potential recordings are shown at 10 mm/s on scale paper. Three panels illustrate baseline (left), and two time points (10 and 17 min) after the higher dose of K201. At 10 min (middle panel), the first pro-arrhythmic activity in the form of single and multiple ectopic beats was observed. Please note that the ectopic activity arises from within the monophasic action potential duration. Later in time, Torsade de Pointes appeared (right panel). The numbers depict RR and QT intervals and the duration of the left and right ventricular monophasic action potential at 90% repolarization.

Table 4. Proarrhythmic effects of dofetilide (experiment 2) and preventive effects of a pretreatment of K201 lower and higher dose (experiments 3a/3b) in dofetilide-susceptible chronic AV-block dogs.

	Baseline 1	Baseline 2	Dofetilide	K201 + Dofetilide
K201 lower (n=6)				
Inducibility, % (95%-CI)	0 (0-44)	0 (0-44)	100	83 (42-99)
TdP, n	0	0	8 ± 3	7 ± 7
T/TdP, s	no TdP	no TdP	11 ± 7	6 ± 6
Defibrillations, n	no TdP	no TdP	6 ± 4	2 ± 3
MEB, n	0	0	22 ± 29	44 ± 56
SEB, n	0	1 ± 2	58 ± 66	52 ± 53
K201 higher (n=3)				
Inducibility, % (95%-CI)	0 (0-62)	0 (0-62)	*100*	100 (38-100)
TdP, n	0	0	7 ± 5	5 ± 3
T/TdP, s	no TdP	no TdP	8 ± 5	28 ± 45
Defibrillations, n	no TdP	no TdP	2 ± 3	3 ± 5
MEB, n	0	0	32 ± 43	37 ± 36
SEB, n	0	0	97 ± 81	100 ± 74

Baseline 1, the interval before start of dofetilide in experiment 2; Baseline 2, the interval before start of K201 pretreatment in experiment 3a or 3b; Dofetilide, the interval after start of infusion of dofetilide in experiment 2; K201 + Dofetilide, interval from start of infusion of dofetilide after K201 pretreatment in experiment 3a or 3b. Dofetilide-susceptibility was assessed in experiment 2; due to the selection inducibility is 100% in the dofetilide group. Please note that no significant differences were detected (used statistics: paired t-test, Baseline 1 vs. Baseline 2 and Dofetilide vs. K201 + Dofetilide). Abbreviations are explained in *table 3*.

completed. In these dogs, no preventive anti-arrhythmic effects were noted: three of three dogs showed repetitive Torsade de Pointes after administration of dofetilide (*Table 4*, lower part).

DISCUSSION

Our results can be summarized as follows: In the chronic atrioventricular block dog, K201 (1) prolonged atrial and ventricular repolarization (dose-dependently), (2) had no significant anti-arrhythmic effects against dofetilide-induced torsade de pointes, and (3) only at the higher dose, increased short-term variability of left ventricular monophasic action potential duration and resulted in torsade de pointes in a minority of animals.

Prolongation of repolarization

The measured steady state plasma concentrations of K201 (*Figure 2*) are in line with those reported in other studies: about 300 ng/ml after the lower dose[6,23] and 700-900 ng/ml with the higher

dose.[10] Without considering protein binding, these values translate roughly into 0.7 μM and 1.5-2.0 μM, respectively (molecular weight of K201 is 461.07), thus close to the IC_{50}s of many of the ion currents blocked by K201 (see introduction). Therefore, it is hard to predict the exact effects of K201 on atrial and ventricular repolarization, as the duration of the cardiac action potential is the result of a complex dynamic system involving numerous in- and outward ion currents.

Both in the control and in the chronic atrioventricular block dog, K201 delayed repolarization. Although these effects occurred earlier (15 min) and persisted longer with the higher dose (*Table 1*), there was no clear dose-dependent finding in normal hearts with the exception of right atrial monophasic action potential duration. In remodeled hearts, dose-dependency of K201 was clearly seen (*Figure 3*). Prolongation of atrial and ventricular repolarization has been described by others too using a dose similar to our higher dose: increases of atrial effective refractory period and QTc in sinus rhythm dogs[10] and of QTc in rabbits.[14] Differential effects on atrial and ventricular repolarization times ('atrial specificity'), as suggested from studies in guinea pigs[9,13] were not observed in this study.

No relevant anti-arrhythmic effects against dofetilide-induced Torsade de Pointes

Intracellular calcium handling is a complex, fundamental process for the proper function of excitation-contraction coupling in cardiomyocytes. Various pathophysiological conditions exist where dysfunction of calcium handling is linked to cardiac arrhythmias, including inherited catecholaminergic polymorphic ventricular tachycardia and heart failure.[5,24-28] Triggering of these arrhythmias may lie in a diastolic calcium leak from the sarcoplasmic reticulum that could activate the transient inward current of the Na^+/Ca^{2+} exchanger, generating delayed afterdepolarizations and possibly (runs of) ventricular triggered beats. K201 has been implicated to provide stabilization of FKBP1B, thereby preventing diastolic Ca^{2+} leak from the sarcoplasmic reticulum and associated ventricular tachycardias.[6]

A second mechanism for triggered arrhythmias is the initiation of early afterdepolarizations: a prolonged action potential and abnormal Ca^{2+} handling may provide a second depolarization during phase 2 or 3 of the action potential, most likely through window currents. In the canine chronic atrioventricular block model, both delayed and early afterdepolarizations are well documented.[29-32] The antiarrhythmic properties of K201 against Torsade de Pointes are therefore of interest mechanistically.

Quantification of dofetilide-induced Torsade de Pointes has shown that the inducibility over weeks is reproducible and the severity stably present for 20 min.[19] The latter allows antiarrhythmic drug testing in the second 10 min window. Thus, both the suppressive properties of K201 could be elucidated, between 10 and 20 min after dofetilide (experiment 2), and its preventive potential against dofetilide-induced Torsade de Pointes (experiments 3a/b). Independent of dose, K201 was neither able to suppress nor to prevent Torsade de Pointes in this model. Its lack of antiarrhythmic potential is in contrast with the results obtained in the alpha-adrenoceptor agonist methoxamine sensitized rabbit study. There, K201 in a much higher dose (13 times our higher

dosage: 400 mg/kg/min for 30 min) was shown to be very effective against clofilium-induced torsade de pointes.[14] As mentioned, a confounding variable in this rabbit model is the alpha-adrenoceptor antagonism effect of K201.[2]

In speculation, this lack of antiarrhythmic effect may argue against a primary involvement of the ryanodine receptor–FKBP1B complex in the generation of ectopic beats and the induction of drug-induced torsade de pointes in this model. Caution is however needed, because K201 (a) has additional effects, including inhibition of I_{Kr}, I_{K1} and I_{Na},[12,13] and (b) intracellular calcium overload has numerous effectors, and stabilizing effects against one may not be sufficient to overcome the others. The latter has been demonstrated in knock-in ryanodine receptor 2 R4496C mice that were not protected by K201 (1 and 10 μM) against isoproterenol-induced delayed afterdepolarizations and ventricular tachycardia.[23]

The lack of antiarrhythmic effects was also surprising as the block of $I_{Ca,L}$ and late I_{Na} are known to be antiarrhythmic in the chronic atrioventricular block model.[33,34] An example is the drug verapamil which shares some similarities with K201 regarding block of membrane ion currents.[33,35] Verapamil, however, did not induce Torsade de Pointes in the chronic atrioventricular block model, rather it was able to completely prevent and suppress dofetilide-induced Torsade de Pointes.

Pro-arrhythmic signals of K201

The anesthetized dog with chronic atrioventricular block is very sensitive to drug-induced Torsade de Pointes:[19] class III drugs with a clinical Torsade de Pointes incidence that varies from 2 to 5%,[36] cause Torsade de Pointes in 70-80% of chronic atrioventricular block dogs.[37,38] On the other hand, the model also shows specificity as evidenced by the fact that a number of drugs do not induce Torsade de Pointes despite (severe) prolongation of QT interval (*Figure 5*). Based on previously published studies of K201, we did not anticipate any pro-arrhythmic effects of K201 despite the fact that K201 is able to prolong repolarization (QTc).[10,14] With the lower dose, this assumption was confirmed. However, the higher dose of K201 revealed several signs of pro-arrhythmia: (1) reproducible Torsade de Pointes induction in one animal (1/7 = 14%), (2) pacing induced torsade de pointes in two more animals, and (3) a significant increase in short-term variability of left ventricular monophasic action potential duration (*Table 2*). When comparing the higher dose of K201 to dofetilide, it is evident that Torsade de Pointes incidence is much lower (1/7 vs. 7/7 or 9/10).

Recently, it was proposed that short-term variability of left ventricular monophasic action potential duration could predict pro-arrhythmic properties of drugs by demonstrating a sudden increase prior to arrhythmogenesis, whereas drugs less likely to cause torsade de pointes did not change the short-term variability.[19,33] The increase seen after the higher dose of K201 is suggestive of proarrhythmic properties.

In the past, programmed electrical stimulation protocols have been used in different studies to increase Torsade de Pointes incidence in this model. An example is d-sotalol (2 mg/kg) that had

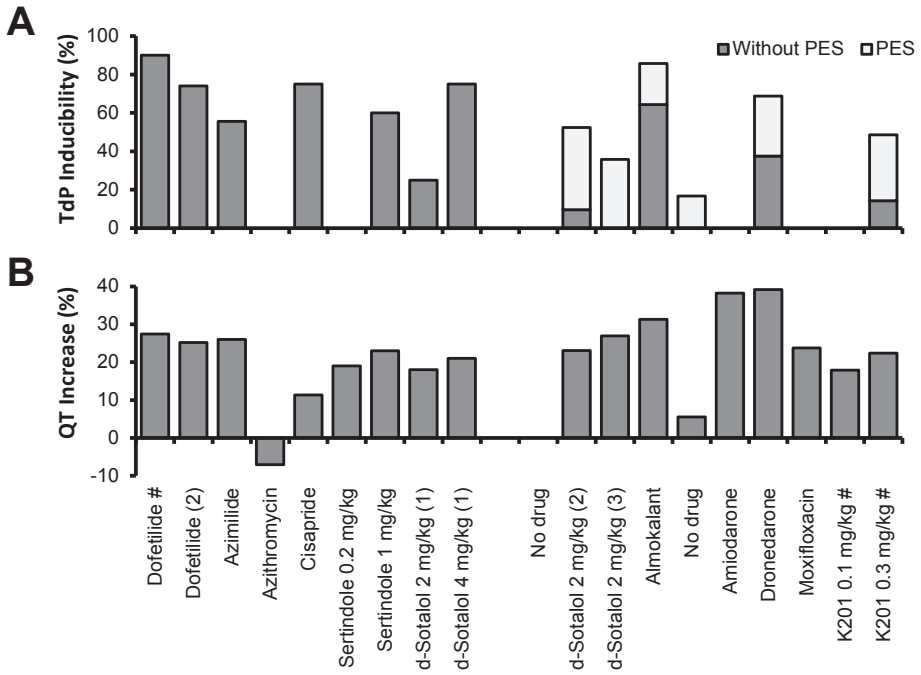

Figure 5. Overview of a number of drugs that have been evaluated for pro-arrhythmic properties in the anesthetized chronic atrioventricular block dog model

A: Spontaneous inducibility of Torsade de Pointes (≥ 3 times) after drug administration (grey bars) ranges from 0 to 90% depending on the drug and the administered dose. On the right side, the contribution of programmed electrical stimulation (PES) to Torsade de Pointes inducibility is shown (white bars). The rate changes induced by programmed electrical stimulation enhance the susceptibility to (drug-induced) Torsade de Pointes considerably.

B: The effects of these drugs on QT interval are presented as relative increase of the mean. #, data from present study. From left to right, the numbers of dogs and the references of the studies are: dofetilide # (n=10), dofetilide[16] (n=27), azimilide[37] (n=9), azithromycin[41] (n=5), cisapride[42] (n=4), sertindole[43] 0.2 mg/kg (n=5) and 1 mg/kg (n=5), d-sotalol[21] 2 mg/kg(1) (n=8) and 4 mg/kg (n=8); no drug (baseline) and d-sotalol 2 mg/kg(2)[39] (n=18); d-sotalol[38] 2 mg/kg(3) and almokalant[38] (n=14, in serial experiments); no drug (n=6), amiodarone (n=7) and dronedarone[44] (n=8); moxifloxacin[41] (n=6); K201 0.1 mg/kg # (n=7) and 0.3 mg/kg # (n=7). All drugs were administered i.v. except amiodarone and dronedarone, which were administered chronically per os.

a low torsade de pointes incidence in the absence of pacing (0-25%),[21,38,39] but with programmed electrical stimulation this value increases to approximately 45% of the animals (*Figure 5*). A similar observation was made with almokalant, from nine of fourteen to twelve of fourteen animals.[38] The Torsade de Pointes incidence of the higher dose of K201 resembles the results obtained with d-sotalol (2 mg/kg). Because d-sotalol and almokalant were never serially compared to dofetilide (to assess susceptibility to drug-induced torsade de pointes), we cannot rule out that interindividual differences in group assembly might be responsible for an overestimation of Torsade de Pointes after the higher dose of K201.

Clinical implications

Although the lower dose of K201 did not increase atrial repolarization duration (right atrial monophasic action potential duration at 50% repolarization), it did increase atrial effective refractory period, which could confer an antiarrhythmic effect on atrial fibrillation or other atrial arrhythmias. Further, the effects of K201 on intracellular Ca^{2+} cycling could have novel antiarrhythmic effects in atrial fibrillation.[9,11] K201 is currently in development for the treatment of atrial fibrillation in humans. It is clear that K201 at the higher dose showed a proarrhythmic signal, suggesting that there may be a dose-dependent potential for K201 to produce clinical Torsade de Pointes. Further studies will be required to reveal both the efficacy and clinically required doses of K201 for treatment of atrial fibrillation and the anti- and pro-arrhythmic effects of this agent on Torsade de Pointes in other model systems and in men, where affinity for various receptors and protein binding of K201 could be different. Also the known existence of metabolites of K201 may beneficially alter the electrophysiological effects and clinical application.[40]

Study limitations

The canine chronic atrioventricular block model is very sensitive to drug-induced Torsade de Pointes, but there is large interindividual variation ranging from non-susceptibility to sudden cardiac death without administration of any proarrhythmic drug. Extrapolation of the observed proarrhythmic signals of K201 to liability for arrhythmias in the human population is therefore uncertain. This may also apply to beat-to-beat variability of repolarization.

Conclusions

Class III effects of K201 were documented, with evidence for modest pro-arrhythmic potential at the higher dose, linked to an increase in beat-to-beat variability of repolarization. No relevant antiarrhythmic effects against drug-induced torsade de pointes were seen in susceptible dogs with chronic atrioventricular block.

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Chapter 3

Commentary

Verapamil as an antiarrhythmic agent in congestive heart failure: hopping from rabbit to human?

Thom R.G. Stams[a], Vincent J.A. Bourgonje[a], Marc A. Vos, Marcel A.G. van der Heyden[1]

Department of Medical Physiology, Division Heart & Lungs, University Medical Center Utrecht, Utrecht, The Netherlands

[a] Both authors contributed equally as first authors

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ABSTRACT

Repolarization dependent cardiac arrhythmias only arise in hearts facing multiple 'challenges' affecting its so-called repolarization reserve. Congestive heart failure (CHF) is one such challenge frequently observed in humans and is accompanied by altered calcium handling within the contractile heart cell. This raises the question as to whether or not the well known calcium antagonist verapamil acts as an antiarrhythmic in this setting, as seen in arrhythmia models without CHF. According to the study of Milberg et al. in this issue of BJP the answer is yes. The results of this study, using a rabbit CHF model, raise important questions. First, given that the model combines CHF with a number of other interventions that predispose towards arrhythmia, will similar conclusions be reached in a setting where CHF is a more prominent proarrhythmic challenge; second, what is the extent to which other effects of calcium antagonism can limit this pharmacological approach as viable clinically in CHF? In vivo studies in large animal CHF models are now required to further explore this interesting, but complex, anti-arrhythmic approach.

Keywords: Heart Failure; Torsade de Pointes; arrhythmia; calcium channel; verapamil; rabbit model; repolarization reserve

In cardiac ventricular myocytes, an imbalance between inward and outward currents may prolong action potential duration. This makes the heart vulnerable to the occurrence of so-called Torsade de Pointes (TdP) arrhythmias which are life threatening ventricular tachycardias that create rapid fluctuations of QRS complexes around the iso-electric line on the human electrocardiogram. In patients, several factors are known independent risk factors or 'challenges' for TdP arrhythmias, including hypokalemia, bradycardia, genetic and drug-induced long QT-syndromes and chronic congestive heart failure (CHF).[1] A single 'challenge' on cardiac ventricular repolarization, for example reduction of a single membrane ion current, usually does not result in repolarization-dependent arrhythmias. Apparently the heart has a reserve, commonly referred to as 'repolarization reserve',[2] and multiple challenges are therefore usually required in order to provoke arrhythmia. Often QT-prolonging drugs associated with TdP arrhythmias are the final challenge that exceeds the reserve, resulting in proarrhythmia. Quantification of the repolarization reserve however, remains difficult. Although a number of surrogate parameters have been suggested,[3] like temporal or spatial dispersion of action potential duration, optimal quantification of repolarization reserve still requires testing of susceptibility to arrhythmias, where the cumulative severity of the challenges required to exceed the reserve then provides an estimation of the reserve. Interestingly, some drugs, including those that block the inward L-type calcium current (I_{CaL}), have been shown to be effective against drug-induced arrhythmias, by counteracting one or more of the predisposing challenges (e.g. Oros, et al.[4]).

Only a few experimental large animal models mimicking CHF have been developed. Currently, the efficacy in which I_{CaL} inhibition prevents or suppresses early after depolarizations (EAD) and polymorphic ventricular tachycardia in CHF is not clear and difficult to predict since calcium handling disturbances are apparent in this disease.[5] Moreover, in a setting of CHF, this apparent simple anti-arrhythmic approach has to deal with conflicting imperatives like anti-arrhythmic action versus hemodynamic tolerance. In this issue Milberg et al. report the outcome of I_{CaL} block by verapamil, a renowned antiarrhythmic compound, on arrhythmic endpoints in a rabbit model of non-ischemic CHF with long-QT characteristics.[6] CHF was generated by continuous right ventricular rapid pacing, where after Langendorff perfused sham and CHF hearts were subjected to a number of additional challenges in order to provoke arrhythmias: bradycardia, ectopic ventricular activation, severe hypokalemia and erythromycin mediated I_{Kr} block. Repolarization was prolonged to some extent in CHF but spatial dispersion was not affected at baseline. Only after I_{Kr} block, especially transmural dispersion was increased to a larger extent in CHF. Arrhythmias were observed, but their number in hearts from sham animals (4 of 11 hearts; 36%) was not significantly different from rabbit hearts with CHF (8 of 11; 73%; p=NS). Unfortunately, surrogate parameters were only reported for normokalemic circumstances when the repolarization reserve is challenged less severely and thus cannot directly be associated with the arrhythmic endpoint. Remarkably, and in favour of the CHF model used here, are the findings of the same group published recently,[7] in which the rabbit hearts were used to analyse the proarrhythmic effect of the I_{Kr} blocker sotalol. In this CHF group, sotalol induced EADs (as estimated from monophasic action potential morphology) and TdP in 16 of 18 (89%) hearts versus 7 of 14 (50%) hearts in the sham group. When we solely compare arrhythmia incidence based on these numbers, a

P-value of 0.023 is obtained (two-tailed Fisher exact test). However in both studies, and yet another (7 of 14 (50%),8 the pronounced incidence of arrhythmias in the sham hearts represents a potential limitation. Nevertheless, verapamil was demonstrated to be an efficient antiarrhythmic in this setting and, importantly, we may thus conclude that effectiveness of I_{CaL} block as anti-arrhythmic treatment persists in an isolated rabbit heart model where CHF is added. The next hurdle will be to reach similar conclusions in an in vivo model where CHF is a more prominent proarrhythmic factor.

Mechanisms of the anti-arrhythmic potential of verapamil against repolarization-dependent arrhythmias have been ascribed to shortening of the QT-interval and decreases in beat-to-beat variability of action potential duration,[4,9] and now Milberg et al. show it counteracts spatial dispersion in a CHF heart too. Promising as it seems, verapamil is contraindicated in CHF, especially in cases with severe systolic dysfunction and reduced fractional shortening.[10] Since verapamil inhibits the systolic calcium flux and consequently contractility, it is negative inotropic and this makes verapamil probably a poor choice in the clinic; certainly when considering that the concentration used by Milberg et al. (0.75 μM) is unable to suppress arrhythmias completely. In the in vivo cAVB dog model, verapamil plasma levels of around 0.5 μM clearly were anti-arrhythmic but also lowered left ventricular pressure.[4] Upon titrating verapamil, antiarrhythmic activity could not be observed without a drop in left ventricular pressure.[9] Other calcium antagonists might be a better option, however, and the authors themselves advocate second generation I_{CaL} blockers. Take for instance nifedipine that more strongly affects smooth than striated muscle[11] whereby lowering peripheral resistance will compensate negative inotropy. Still, this may have a major drawback since to preserve blood pressure where contractility is reduced and vessels are dilated, heart rate must increase, which is also unfavourable for an already weakened heart. Obviously, it would be hard to predict the individual effects on vasodilatation and cardiac contractility, and where they would counterbalance each other in a hemodynamically challenged heart under neurohumoral influence. This should be approached experimentally. Furthermore, while inhibiting systolic calcium may be worrisome, in the case of diastolic dysfunction calcium antagonism might be beneficial, by improving coronary flow and muscle relaxation. Since answering these questions is beyond the opportunities offered by the model of Milberg et al., other models should be employed to address these intriguing possibilities.

In conclusion, the study of Milberg et al. demonstrates the efficacy of verapamil as an anti-arrhythmic in the setting of CHF and provides basic science insights into its mechanism of action of reducing spatial dispersion. Future studies are required to further pinpoint the contribution of CHF to arrhythmogenesis in this model, to recapitulate the findings in models where CHF is a more pronounced proarrhythmic challenge, and to validate the antiarrhythmic efficacy, and demonstrate clinical feasibility, of I_{CaL} block in in vivo models of CHF.

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Conflicts of interest

The authors declare they have no conflicts of interest.

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Chapter 4

The electromechanical window is no better than QT prolongation to assess risk of Torsade de Pointes in the complete atrioventricular block model in dogs

Thom R.G. Stams[1], Vincent J.A. Bourgonje[1], Jet D.M. Beekman[1], Marieke Schoenmakers[1,2], Roel van der Nagel[1,3], Peter Oosterhoff[1,4], Jurren M. van Opstal[1,5], Marc A. Vos[1]

[1] *Department of Medical Physiology, Division Heart & Lungs, University Medical Center Utrecht, Utrecht, The Netherlands*

[2] *Gezondheidshuis Stadshagen, Zwolle, The Netherlands*

[3] *Department of Cardiology, St. Antonius Hospital, Nieuwegein, The Netherlands*

[4] *Department of Experimental Cardiology, Academic Medical Center, Amsterdam, The Netherlands*

[5] *Department of Cardiology, Medisch Spectrum Twente, Enschede, The Netherlands*

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ABSTRACT

Background and purpose: The electromechanical window (EMW), the interval between the end of the T-wave and end of the left ventricular pressure (LVP) curve, has recently been suggested as surrogate for Torsade de Pointes (TdP) in healthy animals, whereby a negative EMW (mechanical relaxation earlier than repolarization) after drug administration indicates an increased TdP-risk. The aims of this study were to assess (1) the effect of the ventricular remodeling in the canine chronic, complete atrioventricular block (CAVB) model on EMW; (2) the effect of the I_{Kr}-blocker dofetilide on EMW; (3) the correlation of EMW with TdP-inducibility.

Experimental approach: Our 11 year database of experiments under general anesthesia was reviewed and experiments included if ECG and LVP were recorded simultaneously at spontaneous rhythm. In total, 89 experiments in 44 dogs were appropriate and were used.

Key results: During normally conducted sinus rhythm or acute atrioventricular block, EMW was positive. During CAVB, EMW was decreased to negative values. Dofetilide further reduced EMW before inducing repetitive TdP in 82% of the experiments. However, subclassification into inducible and noninducible dogs revealed no difference in EMW (-185±85ms vs. -205±103ms, respectively). Analysis of the components of EMW revealed that the observed changes in EMW were solely caused by QT prolongation.

Conclusion: In the canine CAVB model, ventricular remodeling and I_{Kr}-block by dofetilide are associated with negative EMW values, but this reflects QT prolongation, and implies that EMW lacks specificity to predict dofetilide-induced TdP.

Keywords: Torsade de Pointes; electro-mechanical window; cardiac safety pharmacology; ventricular remodeling; electrophysiology; chronic AV-block dog; dofetilide

INTRODUCTION

Torsade de Pointes (TdP) is a life-threatening polymorphic ventricular tachycardia with typical twisting of the QRS complexes around the isoelectric line on the surface electrocardiogram (ECG). TdP often occurs in the setting of drug-induced QT prolongation and can be caused by both cardiovascular and noncardiovascular drugs. The incidence of TdP with noncardiovascular drugs is usually low (sometimes less than 1:10 000), which hampers early detection of this severe adverse event in clinical trials.[1,2]

The heart rate corrected QT interval (QTc) is the clinically most used surrogate parameter to assess risk of drug-induced TdP. QTc is also important in safety pharmacology: analysis of the repolarization duration in animal models (ICH S7B) and a thorough QT study in humans (ICH E14) are important parts of the strategy to detect torsadogenic compounds before introduction on the market.[1] Limitation is that QTc-interval is not a good predictor of torsadogenic risk: drugs that prolong the QTc can be free of TdP or even anti-arrhythmic and TdP can also occur in the settings of a short QTc-interval.[3,4]

Recently, Van der Linde, et al.[5] proposed the electromechanical window (EMW) as a new surrogate parameter for drug-induced TdP. EMW represents the interval between the end of the left ventricular pressure (LVP) curve and the end of ventricular repolarization (T wave) and can be calculated by subtracting the QT interval from the interval from QRS onset to the end of the LVP curve (Q-LVPend) (see *Figure 1*). Normally, the left ventricular (LV) contraction ends after repolarization, resulting in a positive EMW, which remains positive under dynamic physiological conditions.[5,6] In anesthetized dogs that were given isoproterenol in the presence of block of the slowly activating delayed rectifier potassium current (IKs) by HMR1556, Van der Linde, et al.[5] elegantly demonstrated that the duration of LVP and QT changed both in opposite directions to create a negative EMW which was related to the induction of TdP arrhythmias. In addition, they showed that TdP could be treated by drugs that make the EMW less negative.

This concept of EMW was suggested to be also usable in guinea pigs to detect pro-arrhythmic effects of other drugs, like blockers of the rapidly activating delayed rectifier potassium current (I_{Kr}) and the L-type calcium current.[7] Under general anesthesia with pentobarbital, incremental dosages of the test compounds were administered. Drugs with a high risk of clinical TdP consistently caused a negative EMW, whereas drugs with no or low risk did not. For example, acute administration of amiodarone caused a greater increase of Q-LVPend than QT, thereby preventing negative EMW values.

In this study and also in another study conducted by Laursen, et al.[8] arrhythmogenesis in the form of early afterdepolarizations (EADs) or TdP was not a (reached) endpoint. However, in the latter study in Langendorff-perfused Göttingen minipig hearts, EMW values remained positive. More recently, Guns, et al.[9] reached the TdP endpoint in the unremodeled guinea pig using a combination of multiple proarrhythmic hits: anesthesia, adrenaline pretreatment, the I_{Ks} blocker JNJ303, the compound of interest and once more adrenaline to trigger TdP. Using this approach they were able to discriminate unsafe drugs from safe ones, at supratherapeutic concentrations.

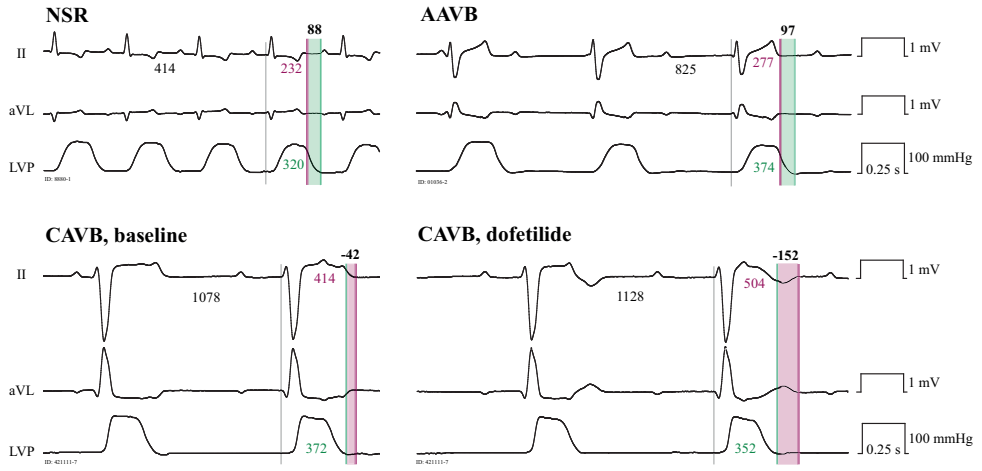

Figure 1

The electro-mechanical window (EMW) in representative experiments at normally conducted sinus rhythm (NSR), acute (AAVB) and chronic atrioventricular block (CAVB), and after dofetilide administration in the same experiment at CAVB. Two ECG leads (II, aVL) and the left ventricular pressure (LVP) signal are shown. Vertical lines represent QRS onset (grey), end of T-wave and the end of the LVP-curve (LVPend). Values (in ms) from top to bottom and from left to right: EMW (bold), RR, QT (magenta) and Q-LVPend (green). At AAVB, EMW was positive (upper panels, shown in green), but at CAVB a prolonged QT interval with unchanged value of Q-LVPend versus AAVB was observed resulting in a negative EMW (lower left panel, red color). Dofetilide further prolonged QT, resulting in an even more negative EMW (lower right panel).

Although the EMW was more negative with the unsafe drugs, the precise contribution of Q-LVPend and QT was not studied and the data suggested a close correlation of EMW with QTc interval.

Ter Bekke, et al.[10] recently applied EMW in anesthetized mongrel dogs with HMR1556-induced long QT1 in which the sympathetic nervous system was stimulated by either right- or left-stellate ganglion stimulation. Interestingly, only left-stellate ganglion stimulation induced TdP and this was linked to a more negative EMW than during right stellate stimulation.

The chronic, complete atrioventricular block (CAVB) model is useful for testing proarrhythmic liability of drugs (review of Oros, et al.[11]). In groups that typically consist of five to ten dogs known torsadogenic drugs were successfully detected with induction of repetitive TdP arrhythmias as primary endpoint. Drugs that are safe despite prolongation of repolarization, e.g. amiodarone[12] were free of TdP in the model, indicating specificity in addition to the high sensitivity (see also overview in Stams, et al.[13]). The increased susceptibility to drug-induced TdP of about 76% with the positive control drug dofetilide, seems to be permanent and serial analysis of inducibility showed high repeatability during the first months after creation of atrioventricular (AV) block, when most experiments are performed in this model.[11,14,15]

In this retrospective study, we addressed the following research questions: (1) what is the effect

of the electrical and contractile ventricular remodeling due to CAVB on the EMW? (2) What is the effect of the I_{Kr}-blocking drug dofetilide on the EMW in the CAVB model? (3) Is the EMW different in TdP-inducible and non-inducible dogs? (4) Does the EMW have additional value over QT for TdP prediction in this model, based on analysis of the effect of these interventions on the electrical and mechanical components of the EMW?

METHODS

A retrospective analysis was performed using the database of all experiments on adult dogs performed by our group, over the period 2000-2011. All these experiments had been performed in accordance to the "European Directive for the Protection of Vertebrate Animals used for Experimental and Scientific Purpose, European Community Directive 86/609/CEE" and with approval from "the Committee for Experiments on Animals" of Utrecht or Maastricht University, The Netherlands.

The following inclusion criteria were used: (1) experiment performed in dogs, by our group at Maastricht or Utrecht University; (2) experiment performed under general anesthesia, using the standard premedication consisting of acepromazine 0.4 mg·kg^{-1} i.m., atropine 0.025 mg·kg^{-1} i.m. and methadone 0.4 mg·kg^{-1} i.m., followed by induction with pentobarbital 25 mg·kg^{-1} i.v. and maintenance with either isoflurane 1.5% or halothane (0.5-1%) in a 1:2 mixture of O_2 and N_2O; (3) simultaneous recording of LVP (Sentron Europe BV, Roden, The Netherlands) and standard 6-lead surface ECG available, and (4) recording available at normally conducted sinus rhythm (NSR), or at idioventricular rhythm acutely after AV-block creation (AAVB) or after at least two weeks AV-block (CAVB).

Exclusion criteria were: (1) cardiac pacing during the experiment that interfered with the measurements of QT or LVP during idioventricular rhythm; (2) Absence of dofetilide (0.025 mg·kg^{-1} in 5 min i.v.) administration in dogs with CAVB, unless serial experiments from the same dog at NSR or AAVB were included. The presence of an endocardial monophasic action potential catheter (EP Technologies, CA, USA) was not an exclusion criterion. Also removal of the LVP catheter before dofetilide administration was not an exclusion criterion.

For a description of the perioperative care, the procedure of AV-block creation and the signal processing we refer to Van Opstal, et al.[16] and Schoenmakers, et al.[15]

Data analysis

Electrophysiological parameters were analysed using the custom made software that was used for the experiment (ECGView, Maastricht, The Netherlands, or ECG-Auto, EMKA Technologies, France or EP Tracer, CardioTek, Maastricht, The Netherlands). At least five consecutive beats were used to calculate the mean for each parameter. QT was measured from the onset of the QRS complex till the end of the T wave in lead II using onscreen callipers and QTC was calculated by

Van de Water's formula.[17] Q-LVPend was measured similarly using the same beats, from the onset of the QRS complex till the end of the LVP curve (i.e. the end of the contractile force). EMW was calculated by subtracting QT from Q-LVPend. The end-diastolic pressure, end-systolic pressure and maximum rise of the LVP per time unit (LV dP/dtmax), a measure of contractility, were also measured using the same software.

Single ectopic beats were defined as ectopic beats initiated before the end of repolarization (T wave) and TdP as a run of 5 or more of such beats with polymorphic twisting of the QRS axis. Measurements after dofetilide were performed at 5 min (i.e. at the moment of complete infusion), provided that TdP was not induced earlier in time and that a window of at least 30 consecutive beats free of ectopic beats was available. Otherwise measurements were performed immediately prior to the onset of ectopic beats or arrhythmias.

In a selected group of inducible CAVB dogs (n=10) an additional determination of EMW just (≤30 sec) prior to the TdP occurrence was performed. The average of five beats was calculated, with exclusion of the two beats immediately after ectopic beats (to limit the influence of the rate acceleration caused by the ectopic beats). In addition, the LVP was meticulously checked for aftercontractions, because of the possible relevance for arrhythmogenesis.

Statistical analysis

Data are expressed as mean±SD. Statistical analysis was performed with the software R (R version 2.15.3, R Foundation for Statistical Computing, Vienna, Austria). A P value <0.05 was considered statistically significant. Paired or unpaired Student's t-test and one-way ANOVA with post hoc analysis with Bonferroni correction were used for analysis.

RESULTS

Screening the database using the inclusion and exclusion criteria as described in the methods, yielded 89 experiments in 44 dogs (Maastricht University: n=20 dogs, 13 female, body weight 26±2 kg, different breeds (mongrel/herding); Utrecht University: n=24 dogs, 14 female, weight 20±3 kg, mongrels from Marshall, USA). These experiments had been performed at NSR (n=21 experiments in 21 dogs), AAVB (n=15 experiments in 15 dogs) and CAVB (n=53 experiments in 34 dogs). The duration of AV-block was 5±4 weeks, ranging from 2 to 15 weeks. Dofetilide was administered at CAVB in 49 experiments in 31 dogs and a recording of LVP was available for analysis during dofetilide in 38 experiments. If the dogs were tested serially, at least two weeks for recovery was present in between the experiments.

A representative registration of ECG and LVP with calculation of EMW, as also described by Van der Linde, et al.[5], is shown in *figure 1*.

Effects of AAVB creation and of remodeling due to CAVB

In dogs with NSR (n=21) the mean cycle length was 583±96 ms (*Table 1*). Creation of AV-block acutely resulted in an altered ventricular activation pattern due to emergence of idioventricular rhythm, with a longer ventricular cycle length of 911±276 ms (P<0.01 vs. NSR). The EMW was not different in dogs with NSR and AAVB and neither the components QT and Q-LVPend (*Table 1*). After remodeling due to CAVB the cycle length was even longer (P<0.001 vs. AAVB) and EMW was decreased (P<0.001 vs. AAVB), which was solely caused by an increase of repolarization duration (QT increased; P<0.001), as Q-LVPend was unchanged .

Effects of dofetilide in CAVB dogs

In CAVB dogs, administration of dofetilide induced repetitive TdP episodes in 40 out of 49 experiments (82%). An individual, representative example of TdP induction by dofetilide is shown in *Figure 2*. Paired analysis comparing the electrophysiological parameters before arrhythmogenesis with baseline revealed that dofetilide further decreased the EMW (P<0.001), fully explained by an increase of QT (P<0.001) as Q-LVPend showed a trend to only a minimal shortening (P=0.053; *Table 2*). Dofetilide also caused an increase of the RR interval and end-systolic pressure and contractility (*Table 2*).

Subgroup analysis based on TdP-inducibility

Stratification based on TdP-inducibility revealed a significant difference in EMW at baseline, before administration of dofetilide in susceptible and nonsusceptible animals (*Table 3*). Analysis

Table 1. EMW determined at NSR, AAVB and CAVB

	NSR	AAVB	CAVB
RR (ms)	583 ± 96**	911 ± 276	1293 ± 357***
EMW (ms)	93 ± 24	80 ± 26	-53 ± 59***
Q-LVPend (ms)	362 ± 23	361 ± 27	361 ± 47
QT (ms)	270 ± 28	281 ± 24	411 ± 71***
QT¬C (ms)	306 ± 24	288 ± 24	385 ± 57***
LV EDP (ms) (mmHg)	5 ± 5*	11 ± 5	8 ± 5
LV ESP (mmHg)	92 ± 20	106 ± 17	106 ± 23
LV dP/dtmax (mmHg·s^{-1})	1473 ± 367	2161 ± 880	2840 ± 1335

NSR, normally conducted sinus rhythm, before creation of AV-block (n=21 experiments); AAVB, acute, complete AV-block (n=15); CAVB, chronic, complete AV-block (n=53); EMW, electro-mechanical window; QTC, heart-rate corrected QT interval using Van de Water's formula; LV, left ventricular; Q-LVPend, interval from begin of QT interval till the end of the LV pressure (LVP) curve; EDP, end-diastolic pressure; ESP, end-systolic pressure; dP/dtmax, maximum rate of LV pressure rise. Values are presented as mean ± SD. */**/***, P<0.05/0.01/0.001 vs. AAVB. Statistics: one-way ANOVA with post hoc Bonferroni test vs. AAVB.

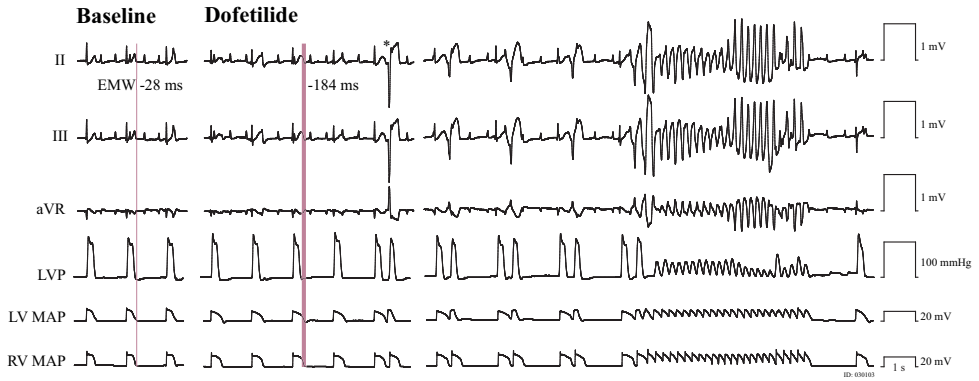

Figure 2. Individual, representative example of arrhythmia induction with dofetilide in a CAVB dog.

Shown are 3 surface ECG leads, the left ventricular pressure (LVP) and left and right ventricular monophasic action potentials (MAP). The left panel shows the baseline values with an EMW of -28. The middle panel shows the measurements after dofetilide, before the first ectopic beat (marked with *) at 3:45 (min:s). Dofetilide decreased the EMW considerably to -184 ms before induction of TdP.

Table 2. Effects of dofetilide in CAVB dog

	Baseline	Dofetilide
RR (ms)	1303 ± 359	1428 ± 372***
EMW (ms)	-53 ± 60	-189 ± 87***
Q-LVPend (ms)	361 ± 48	358 ± 46
QT (ms)	410 ± 73	533 ± 106***
QTc (ms)	384 ± 58	496 ± 86***
LV EDP (mmHg)	8 ± 5	9 ± 6
LV ESP (mmHg)	107 ± 24	114 ± 20***
LV dP/dtmax (mmHg·s^{-1})	2853 ± 1360	3343 ± 1334***

Abbreviations are explained in the legend of *table 1*. Only dogs were EMW was measured serially before and after dofetilide were included for this analysis (n=38 experiments).

***, P<0.001 vs. baseline (paired Student's T-test).

of the components again revealed no shorter Q-LVPend in susceptible dogs, but only a longer QT interval (P<0.01). Analysis of the other parameters also revealed a longer RR interval in the susceptible dogs and a higher LV end-diastolic pressure (P<0.05). After administration of dofetilide, no significant differences were present any more for any of the parameters, including EMW (*Table 3*).

Amiodarone

In the previously published study of Van Opstal, et al.[12] in CAVB dogs (six weeks after AV-block

creation; n=7) chronic treatment with amiodarone (40 mg·kg^{-1} day-1) for four weeks resulted in a significant increase of QT from 340±40 ms to 470±75 ms (P<0.05), but no induction of TdP. Q-LVPend was increased from 337±21 ms to 366±27 ms (P<0.05), but this increase was not enough to prevent a reduction of EMW to negative values (from -5±42 ms to -103±54 ms, P<0.01).

Components of EMW

Simple linear regression analysis after pooling all data from the individual experiments confirmed the close correlation of EMW with QT and the absence of correlation with Q-LVPend interval (*Figure 3*).

Detailed analysis

In ten TdP-inducible CAVB dogs, we extended the EMW determination more closely (0.2±0.2 min) before the occurrence of TdP. In these dogs, the original measurement before the first ectopic beat was performed at t=3.0±1.3 min, whereas the first TdP was seen on average 1.6 min later (range 0.2 to 5.2 min), at t=4.5±2.3 min. The extended analysis did not reveal differences in the parameters (all P>0.1 using a paired student's test): EMW (-151±92 ms before TdP vs. -153±36 ms before the first ectopic beat), QT (489±88 ms vs. 484±45 ms) and Q-LVPend (338±42 ms vs. 332±30 ms).

In only one dog of these dogs, we could detect low amplitude diastolic aftercontractions which were not closely related to TdP. The absence of clear diastolic aftercontractions is also seen in *figure 3*, where ectopic beats within the LV monophasic action potentials occurred.

DISCUSSION

The main findings of this study are the following: (1) Ventricular remodeling due to CAVB is linked to a decrease of EMW to values below zero; (2) In CAVB dogs, dofetilide further decreased EMW to deep negative values, independent of whether TdP is induced or not in the experiment; (3) EMW reflects QT because Q-LVPend was not influenced by either the remodeling or by dofetilide; (4) Although chronic amiodarone treatment resulted in a small increase of Q-LVPend, EMW reached clear negative values, despite absence of TdP induction.

Conditions affecting TdP (may) differ between the models

For use in safety pharmacology, EMW was initially described in adrenergic provoked, tachycardia-dependent, long QT1 circumstances, mimicked by the blockade of I_{Ks} through HMR1556 in normal (unremodeled) anesthetized (fentanyl-etomidate) canines.[5,18] Initiation of TdP was preceded by a shift towards severe negative EMW values that returned to almost control values

Table 3. EMW stratified based on TdP inducibility, at baseline and after dofetilide administration

	Baseline		Dofetilide	
	TdP-	TdP+	TdP-	TdP+
RR (ms)	1127 ± 250	1341 ± 369*	1308 ± 335	1454 ± 378
EMW (ms)	-14 ± 31	-61 ± 62**	-205 ± 103	-185 ± 85
Q-LVPend (ms)	335 ± 55	367 ± 45	341 ± 58	362 ± 43
QT (ms)	348 ± 59	424 ± 69**	507 ± 151	539 ± 95
QT¬C (ms)	338 ± 47	394 ± 56**	480 ± 128	499 ± 76
LV EDP (mmHg)	6 ± 4	9 ± 6*	4 ± 6	10 ± 5
LV ESP (mmHg)	101 ± 31	108 ± 22	107 ± 21	116 ± 20
LV dP/dtmax (mmHg·s-1)	3127 ± 1765	2793 ± 1274	3607 ± 1245	3404 ± 1371

TdP+, repetitive TdP arrhythmias induced by dofetilide; Other abbreviations are explained in table 1. */**, P<0.05/0.01 TdP+ (n=40) vs. TdP- (n=9) (Statistics: Student's t-test). After dofetilide, LVP was recorded in 38 experiments (n=7 and n=31 for TdP- and TdP+, respectively), whereas ECG was available in all experiments.

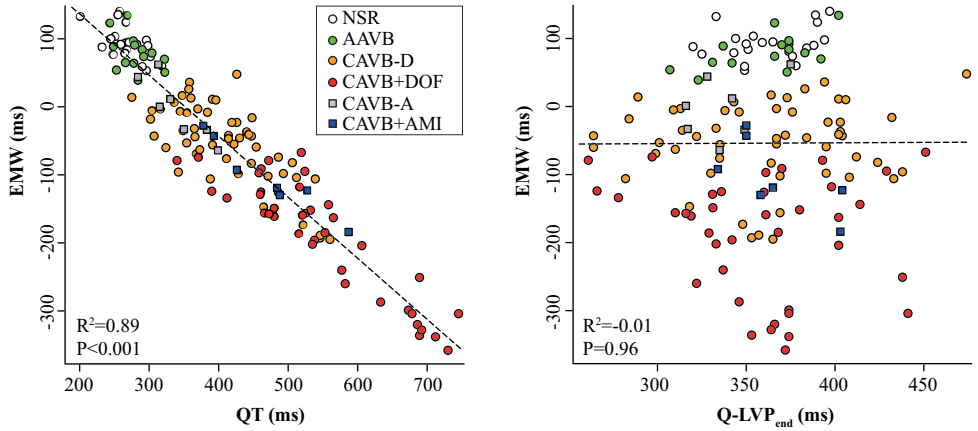

Figure 3

This figure summarizes the contribution of the components of EMW, QT and the interval from QRS onset to end of the LVP curve (Q-LVPend), using the pooled data of all experiments (normally conducted sinus rhythm, acute and chronic AV-block, dofetilide and amiodarone). EMW correlates well with QT (left), with an adjusted R^2 value of 0.89 and slope of -0.90 (95% confidence interval of the slope: -0.96 to -0.85), but not with Q-LVPend (right). This is in agreement with the comparisons of the groups (*Tables 1 and 2*).

Circles: white, normally conducted sinus rhythm; green, acute AV-block; yellow, chronic AV-block at baseline; red, chronic AV-block after dofetilide; Squares: grey, chronic AV-block baseline; blue, chronic AV-block after chronic amiodarone administration.

after anti-arrhythmic therapy with verapamil or atenolol. EMW was changing (becoming negative) due to the fact that the QT time initially was increased after HMR1556 and subsequently Q-LVPend was decreased with the addition of isoproterenol, without further increase of QT,

although QTc (not relevant for EMW calculation) did increase. Thus, important is that QT duration is unable to shorten upon the increasing heart rate after isoproterenol, due to the I_{Ks} block.

The canine CAVB model is not developed to mimic a clinical, monogenetic long QT syndrome, but is a model of acquired long QT. In the model, compensated hypertrophy and numerous adaptations in ion channels and calcium handling proteins have been reported:[11] downregulation of the delayed rectifier potassium currents, increase of the sodium-calcium exchange current and increased calcium content of the sarcoplasmic reticulum, which provides larger calcium transients and an increase of LV contractility parameters.[19-21] Besides the ventricular remodeling, the anesthetic regimen is a key ingredient for TdP induction.[22]

Adrenergic stimulation and increasing heart rate, like applied in the other studies of EMW, is not part of the standard methodology to induce TdP in the CAVB model, but sudden rate accelerations by programmed electrical stimulation have been applied to increase the inducibility of drug-induced TdP.[13,23] In contrary, single injections of adrenaline did not increase inducibility and continuous pacing at a higher basic heart rate and isoproterenol administration have been applied to reduce the incidence of TdP.[12,23,24]

Mechanisms of TdP

Extensive research has been performed to elucidate the underlying mechanisms of TdP, but the arrhythmogenesis of TdP in general and in the CAVB dog are still not fully understood. EADs and delayed afterdepolarizations (DADs) are considered the primary cause of focal activity responsible for the initiation of the arrhythmias, although re-entry may also be involved especially during the perpetuation.[4,25]

In the CAVB dog, antiarrhythmic effects against TdP have been demonstrated by inhibition of EADs or DADs, for example using the calcium channel antagonists verapamil and flunarizine.[21,26] In a recent study, the anti-arrhythmic agents verapamil and SEA-0400 (n=3 for each drug) successfully suppressed dofetilide-induced TdP in this model.[27] This effect was neither accompanied by shortening of the QT/QT_C interval,[27] nor by changes of Q-LVPend, thereby resulting in maintenance of a deep negative EMW (-318±17 ms after dofetilide+verapamil and -342±49 ms after dofetilide+SEA-0400).

The initiation of DADs has been related to spontaneous calcium release from the sarcoplasmic reticulum during diastole. The distinction between DAD and EAD is only based on appearance in time: within the repolarization (action potential) or not, so not based on their mechanism. Therefore, they may share the same mechanism. In a long QT2 rabbit model, oscillations in intracellular Ca^{2+}, most likely caused by spontaneous Ca^{2+} release from the sarcoplasmic reticulum, preceded the oscillations in membrane potential and EADs and seemed to be driven by the increase of intracellular Ca^{2+}.[28] However, in the setting of a prolonged repolarization duration, window currents, especially of the L-type calcium current provide an additional mechanistic explanation for EADs in phase 2, but also in phase 3 of the action potential.[29,30] The window currents are enhanced in left ventricular cardiomyoctes from CAVB dogs and are increased further

by beta-adrenergic stimulation.[31] Only recently, distinctions between sarcolemmal and sarcoplasmic reticulum dependent EADs have been documented by difference in rate and their response to drugs and dynamic action potential clamp.[30] However, these researchers also postulated that these mechanisms may act synergistically.

Aftercontractions and spontaneous Ca^{2+} release

In the intact heart, the LVP can be used to time-dependently relate mechanical information to electrical effects and ectopic activity. Intriguing was the observation by Gallacher, et al.[18] that mechanical diastolic aftercontractions in the LVP seemed to precede EADs in the monophasic action potential recordings prior to TdP occurrence, suggesting that these aftercontractions may be caused by spontaneous Ca^{2+} release from the sarcoplasmic reticulum. Due to the negative EMW these diastolic aftercontractions do not induce DADs but EADs in the action potentials and when triggering: ectopic beats within the QT interval, the R-on-T phenomenon.

A limitation is that in cells DADs or EADs can be relatively easily visualized, whereas this is much more complicated in the intact animal: a monophasic action potential is only recorded locally and restricted in its visualization because of electrical coupling of the myocytes. Due to the source-sink relations, spatiotemporal synchronization of EADs or DADs is required before afterdepolarizations can be visualized and focal activity can be induced and additional factors including adrenergic stimulation can influence this process.[32]

The aftercontractions also raise the interesting possibility of mechano-electrical feedback via stretch-sensitive ion currents as a cause of the EADs and arrhythmogenesis of TdP.[33]

Implications for application of EMW in safety pharmacology

As described earlier, we found no clear additional value of EMW over QT in the CAVB model as surrogate for drug-induced TdP and the measured QT prolongation after drug administration is not well associated with TdP-inducibility.[4,12,13,34] As a consequence, other parameters may be more useful, obviously the arrhythmogenic outcome itself, but also other surrogate parameters. Especially noteworthy is beat-to-beat variability of repolarization, quantified as short-term variability of the LV monophasic action potential duration, because this parameter has been reported to be superior to QT and QTc in the CAVB model, as reviewed by Oros, et al.[11] and Varkevisser, et al.[35] Also cardiac wavelength, triangulation, reverse use dependence, instability and dispersion (λ-TRIaD) have an advantage over QTc, which is described in the editorial of Hondeghem.[3]

The apparent lack of specificity of EMW does not contradict the role of EMW to depict the substrate. TdP was consistently preceded by negative EMW values. This is in accordance with the other models and Ter Bekke and Volders[33] already discussed that EMW most likely is not the 'primum movens.' However, our results implicate that a negative EMW is not always proarrhythmic and for safety pharmacology purposes, other parameters related to the actual triggering of arrhythmias episodes need to be studied as well.

Study limitations

The relevance of the EMW was studied in this particular canine model of dofetilide-induced TdP under our standard conditions, which may limit extrapolation to other models and humans: (1) the used anesthetics prolong repolarization duration and negatively affect the left ventricular pressure; (2) the origin of idioventricular rhythm is not controlled, leading to different activation patterns; (3) the degree of bradycardia after AV-block is not controlled; (4) reproducibility in the serial experiments is not complete, precluding 100% segregation in inducible and non-inducible animals. Only two drugs were studied for proarrhythmia: dofetilide (our gold standard to induce TdP) and amiodarone, which causes QT prolongation without induction TdP.

We did not study the effect of dofetilide on the EMW at AAVB, because a LVP recording during dofetilide was only available in one experiment, in which EMW remained positive. TdP was not induced, which is in agreement with earlier experiments.[36] This study has a retrospective design, which may be more susceptible to bias, although only data from prospectively performed studies were used, with similar offline analysis of the data. Due to the limited amplitude resolution of the recording system on-screen and the presence of AV-block (interference by P waves) reliable observation of aftercontractions was only possible if the amplitude exceeded about 10 mmHg.

Conclusions

In the canine CAVB model, ventricular remodeling and I_{Kr}-block by dofetilide are associated with negative EMW values, but this closely reflects QT prolongation. We found no difference in the EMW after dofetilide between experiments with and without TdP arrhythmias. Chronic amiodarone treatment also decreased the EMW to negative values, despite the absence of TdP arrhythmias. Therefore, we conclude that in the CAVB model TdP arrhythmogenesis is linked to negative EMW values, but the effects of dofetilide and amiodarone imply that EMW has the same limitation as QT: it lacks specificity to predict drug-induced TdP in the canine CAVB model.

Conflicts of interest

None.

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Chapter 5

Chronic bradycardic right ventricular apical pacing is proarrhythmic, but the electrical remodeling is disguised

Thom R.G. Stams[1], Jet D.M. Beekman[1], Roel van der Nagel[1,2], Jacques M.T. de Bakker[1,3], Peter Loh[4], Marti F.A. Bierhuizen[1], Bart Kok[1], Marc A. Vos[1]

[1] *Department of Medical Physiology, Division Heart & Lungs, University Medical Center Utrecht, Utrecht, The Netherlands*

[2] *Department of Cardiology, St. Antonius Hospital, Nieuwegein, The Netherlands*

[3] *Interuniversity Cardiology Institute of the Netherlands*

[4] *Department of Cardiology, Division Heart and Lungs, University Medical Center Utrecht, Utrecht, The Netherlands*

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ABSTRACT

Introduction: In the canine chronic, complete atrioventricular block model, both chronic bradycardia and abnormal ventricular activation due to idioventricular rhythm contribute to the proarrhythmic left ventricular (LV) electrophysiological remodeling and the associated, enhanced susceptibility to dofetilide-induced Torsade de Pointes (TdP). In this study, the effect of chronic, bradycardic RV apical (RVA) pacing on LV electrophysiological remodeling and TdP susceptibility was investigated.

Methods: In eight anesthetized dogs, RVA pacing at lowest captured rate was initiated after creation of AV-block. At a fixed pacing rate of 60/min, ECG parameters, LV and RV monophasic action potential duration (MAPD) and short-term-variability of LV MAPD (STV_{LV}) were measured acutely and after at least three weeks of remodeling. During the latter experiment, dofetilide was administered to study inducibility of TdP. In two additional dogs, mapping of activation recovery intervals (ARIs) was performed, to evaluate intraventricular heterogeneity of repolarization and the relation with arrhythmogenesis.

Results: LV and RV MAPD and STV_{LV} were not increased significantly after remodeling; only QT interval tended to increase (+11%, P=0.06). However, dofetilide-induced TdP occurred in 6 out of 8 dogs (75%), which was associated with large changes in electrical parameters. Mapping data revealed that ARI prolongation preferably occurred in the early activated areas. Dofetilide further increased ARI heterogeneity and resulted in induction of ectopic beats, preferentially at a n LV site with large local spatial dispersion of repolarization moments.

Conclusion: Based on commonly used electrophysiological parameters at baseline, chronic bradycardic RVA pacing was not associated with development of significant electrophysiological remodeling, based on analysis of commonly used electrophysiological parameters. However, dofetilide induced TdP in most animals. The induction of ectopic beats was associated with large local LV spatial dispersion of repolarization moments.

Keywords: Artificial cardiac pacing; Remodeling; Torsade de Pointes; Ventricular arrhythmias; Electrophysiology; Repolarization; Dispersion

INTRODUCTION

For decades, the RVA has been used as the standard place of the pacing electrode for ventricular pacing. Reasons for this include the clinical experience, feasibility of the lead implantation procedure and the long-term lead/pacing stability, whereas complications including lead dislodgement/failure, diaphragmatic stimulation and cardiac perforation are relatively rare.[1]

Although RVA pacing can be beneficial for patients with symptomatic bradycardia (for example due to third degree AV-block), it is now clear that RVA pacing has adverse effects on LV function and can even lead to heart failure in a minority of patients. This is particularly related to the activation pattern, which resembles left bundle branch block (LBBB), a dyssynchronous LV activation pattern that results in LV mechanical dyssynchrony.[2-6] Studies in humans and animals have shown that besides an acute reduction of pump function, long-term adverse effects of dyssynchronous LV activation include ventricular remodeling (hypertrophy, dilatation), changes in perfusion and metabolism, but mostly a compensated state (preservation of LV function).[7-11] Ventricular pacing from the RVA is associated with a higher risk of atrial fibrillation compared to atrial based pacing, but the consequences of RVA pacing for arrhythmogenesis of repolarization dependent ventricular arrhythmias are largely unexplored.[6]

Chronic complete atrioventricular block (CAVB) causes ventricular remodeling and is a known risk factor for drug-induced Torsade de Pointes (TdP). The anesthetized, canine CAVB model has been used extensively as a model to study drug-induced TdP. Infusion of dofetilide, a specific blocker of the rapidly activating delayed rectifier potassium current (I_{Kr}), into these dogs results in induction of repetitive TdP episodes in about 75% of the dogs and is therefore used as reference for this study.[12]

Originally, the increased susceptibility to TdP in CAVB dogs has been explained by ventricular remodeling as a consequence of the bradycardia-induced volume overload. Later, a role for the abnormal ventricular activation pattern was implicated.[13] Compared to a group of unpaced AV-block dogs with idioventricular rhythm, AV-block dogs subjected to chronic bradycardia, but a more physiological activation by high-septal pacing (HSP), showed attenuated ventricular electrical remodeling. The latter was based on electrophysiological parameters and was linked to a decreased severity of dofetilide-induced TdP.

In this study, we investigated the combined effect of bradycardia and dyssynchronous ventricular activation (DVA), established by chronic right ventricular apical (RVA) pacing at lowest captured rate, on ventricular electrical remodeling and TdP susceptibility. Although conflicting reports exists on this topic, building on the study of Winckels,[13] we hypothesized that during RV apical pacing, the late left ventricular (LV) activation would result in attenuation of LV electrical remodeling and thereby prevention of proarrhythmia.

METHODS

All experiments were approved by the Committee for Experiments on Animals of Utrecht University, Netherlands and in accordance with the "European Directive for the Protection of Vertebrate Animals used for Experimental and Scientific Purpose, European Community Directive 86/609/CEE". A standard 6-lead surface electrocardiogram (ECG) was recorded at sinus rhythm to exclude significant pre-existent ECG abnormalities.

Anesthesia and perioperative care

Experiments were performed under general anesthesia. Thirty minutes after premedication (methadone 0.5 mg/kg, acepromazine 0.5 mg/kg and atropine 0.5 mg i.m.) anesthesia was induced with pentobarbital sodium 25 mg/kg i.v. and maintained by isoflurane 1.5% in O_2 and N_2O, 1:2. The dogs were endotracheally intubated and mechanically ventilated, with a fixed respiratory rate of 12-14/min, while tidal volume was adjusted to maintain the end-tidal carbon dioxide concentration between 3.5 and 4.5%. Preoperatively, ampicillin 1000 mg i.v. was administered as antibiotic prophylaxis. Postoperatively, dogs received ampicillin 1000 mg i.m. and the analgesic buprenorphine 0.3 mg i.m. The femoral artery and vein, or carotid artery or jugular vein, were dissected to insert the required catheters, including monophasic action potential (MAP) catheters (Hugo Sachs Elektronik, Germany). A standard 6-lead surface ECG and four precordial leads (LL, LU, RL and V10) were recorded during the experiments.

Study design

For this prospective study with three serial experiments, adult purpose bred dogs of either gender (mongrel, Marshall, USA, n=9 or Beagle from Harlan, Denmark, n=2; nine females; body weight 21±2 kg) were initially included in the study. In an additional (pilot) study, two dogs (mongrel, Marshall, USA) were added to study intraventricular differences of repolarization by endocardial (catheter) and transmural (needle electrodes) mapping.

Acute bradycardic RVA pacing

Via the jugular vein, a pacemaker lead (screw-in, steroid-eluting, bipolar lead 5076, Medtronic) was positioned in the RVA under fluoroscopic guidance. The lead was connected to a pacemaker (Vitatron or Medtronic, different models) which was implanted subcutaneously in the neck or, after extending and tunneling of the lead, at the thorax. The threshold was set to at least two times the capturing threshold, with a minimum of 2.5 V and pulse duration of 0.5 ms. Proximal His bundle ablation was performed by catheter ablation, at a power of 30 W for 30 s to create irreversible, third-degree AV-block.[14] After at least five minutes RVA pacing in VVI mode at a rate of 60 bpm (or 70 bpm if spontaneous idioventricular rhythm resulted in noncapture or fusion), MAP signals were recorded at the apical free wall of the LV and RV, at least 2 cm away from the

pacing electrode.

Three out of eleven dogs were lost to follow-up: one dog had died during the electrophysiological measurements in the first experiment due to a complication of the catheterization (retroperitoneal bleeding due to vascular perforation) and two other dogs were excluded owing to recurrent pacing lead dislodgements. In the eight remaining dogs, pacing lead dislodgement occurred in four animals, requiring a single (n=3) or two (n=1) re-implantations.

Remodeling period

Between the serial experiments, RVA pacing was performed at the lowest captured rate (5-10 bpm faster than the spontaneous idioventricular rhythm), with a minimum of 40 bpm (device limitation). Preference was to obtain at least 95% ventricular pacing; if beneficial, additional settings were programmed, including a lower night than day rate. If pacemaker lead dislodgement occurred, this was followed by at least one week of successful pacing, before the next experiment was performed. The median percentage of ventricular pacing during the remodeling period was 92% (interquartile range 85-93%, range 77-98%) in the eight dogs.

Chronic bradycardic RVA pacing

The second experiment was scheduled three weeks after the first experiment. Recordings at baseline (ECG with LV and RV MAP) were performed, similar to those in experiment 1. Next, dofetilide (0.025 mg/kg/5min i.v.) was infused to test the arrhythmia susceptibility. The infusion was aborted if TdP was induced (defined as a polymorphic ventricular tachycardia with a twisting QRS of at least five beats). Cardioversion (10-150-200 J) was applied if TdP did not terminate spontaneously within ten seconds.

Epicardial MAP recordings and taking of biopsies

Before sacrifice of four dogs, directly after thoracotomy, epicardial MAPs were recorded on the LV and RV, at the apex, midventricular (antero-lateral) level and the base, to study the activation pattern (pacing at 60 bpm). These data showed that the RV apex was activated earliest (32±11 ms), while the midventricular and basal site of the RV were activated later (activation times: 40±17 ms and 48±18 ms, respectively). The activation times of the LV were: 76±9 ms (apex), 80±4 ms (free wall, midventricular level) and 83±4 ms (free wall, near base). Statistically, both the interventricular and the apex-base gradient of the activation times were significant (P=0.027 and P=0.035, respectively), while the interventricular difference was independent of the apex-base level (the interaction was not significant, P=0.18). These data are in agreement with more detailed electrical activation mapping in dogs.[8]

Following these measurements, biopsies were taken and used for analysis of mRNA expression levels (see below).

Real-Time Quantitative PCR (RT-qPCR)

Total RNA was isolated from control dogs with normally conducted sinus rhythm and chronic bradycardic RVA paced dogs (LV mid-anterolateral free wall; n=4 for each) using TRIzol Reagent (LifeTechologies Europe BV, Bleiswijk, Netherlands; #15596-026) according to the manufacturer's protocol. The isolated RNA was subsequently treated with DNAse I (Promega, Madison, WI, USA; #M6101). cDNA was then prepared from 2 µg DNAse I-treated RNA using SuperScript II Reverse Transcriptase (LifeTechologies; #18064-071). For amplification, the cDNA was first diluted 10-fold. TaqMan RT-qPCR assays were performed in 10 µl reactions using TaqMan Gene Expression Master Mix (LifeTechologies; #4369514) and specific TaqMan gene expression assays as listed in *Supplemental Table 1*. Reactions were initiated by a polymerase activation step for 10 min at 95°C. Amplification was obtained in 40 cycles of 15 seconds at 95°C with a 1 minute annealing and extension step at 60°C in a MyiQ2 Real-Time PCR Detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). RT-qPCR assays were carried out in duplo. The hypoxanthine-guanine phosphoribosyltransferase (HPRT1) gene was used for normalization of mRNA levels, since the HPRT1 mRNA levels were not different between the samples in the two groups. The relative difference in expression levels was based on the $2^{-\Delta\Delta Ct}$ method[15], with the adaptation that data are presented as geometric mean with error bars representing geometric standard deviation.

Extensive activation and repolarization mapping in two additional dogs

In the two additional dogs, during the first and second serial experiment, at acute and chronic (four weeks) RVA pacing, electroanatomical mapping was performed (EnSite NavX system, St. Jude Medical, St. Paul, MN, USA). The positions of the EnSite NavX surface electrodes were marked on the skin to facilitate identical placement during the next experiment. A reference screw-in electrode, required for NavX, was temporarily placed at the RV septum. Then, a three-dimensional contour map of the LV and RV cavity was generated using a steerable quadripolar catheter (35G07R, 4mm Celsius A Curve, 6F, Biosense Webster, Diamond Bar, CA, USA). This map was used to track the three-dimensional position of this catheter, to record electrograms at evenly distributed sites within LV and RV (at least 50 sites per ventricle, recording time at least 30 s per location). ECG and electrograms were recorded simultaneously with the Prucka system (Prucka CardioLab, General Electric Medical Systems, Fairfield, CT, USA). The unipolar electrograms, used for calculation of activation recovery intervals (ARIs), were recorded with a sampling frequency of 1 kHz and the following filtering setting: high pass 0.05 Hz, low pass 500 Hz. This procedure was repeated four weeks later, during the second experiment.

After the previous recordings, successful needle mapping was performed in the second animal (referred to as dog #1 in the figures). A thoracotomy was performed to expose the heart. Needle electrodes were inserted in the LV, RV and septum (via the RV), perpendicular to the wall, to record intramural unipolar electrograms. During insertion of the needles the electrogram was monitored to reduce luminal placement of the electrodes (these signals were excluded). The pro-

cedure was similar to a previous study.[16] A total of 57 needles were used and inserted at evenly distributed distances in the LV (24 needles), septum (22) and RV (11). Each needle consisted of four (LV and septum) or two (RV) electrodes with a 4 mm electrode distance. The septal needles were longer to enable insertion via the RV. A schematic overview of the placement of the needles is shown in *Figure 1*. Recordings were performed at baseline and during infusion of dofetilide, with the aim to determine the origin of dofetilide-induced TdP-arrhythmias. All signals were recorded simultaneously with the ActiveTwo system (Biosemi, Amsterdam, Netherlands), which had the following characteristics: sampling frequency 2 kHz, filtering: DC-400Hz (-3dB); digitalization: 24 bit per channel; input range 262 mV to -262 mV.

Analysis

Measurements of the surface ECGs were performed in lead II using calipers (average of 5 beats). QT was measured from the pacemaker stimulus artifact till the end of T wave. QRS was measured form the pacing stimulus artefact till the J point. T wave peak to end interval ($T_{peak\text{-}end}$) was measured from the peak of the T wave till the end of the T wave and used as a measure of global spatial dispersion of repolarization.[17] If the T wave was (initially) negative, the nadir of the T wave was used instead. Durations of LV and RV MAP at 80% repolarization (MAPD) were determined using automated measurements in custom-written Matlab software (Mathworks, Natick, USA). Beat-to-beat variability of repolarization was quantified as short-term variability (STV) of both LV and RV MAPD (STV_{LV} and STV_{RV}), as follows: the absolute difference of two consecutive beats was divided by the square root of 2 and then STV was calculated by averaging 30 consecutive values.[18] Activation times of the MAP were measured from pacing spike until the steepest upstroke of the MAP. ΔAT was defined as the difference between LV and RV activation time. Difference in activation time of ectopic beats (ΔAT_{EB}) was defined as the mean difference between the moment of steepest upstroke of the LV and RV MAP of the first five ectopic beats after start of dofetilide (only including the first ectopic beat in runs consisting of more than one beat). The difference in activation time of TdP (ΔAT_{TdP}) was measured similarly, using the first five beats of the first TdP. These ΔAT measurements were used to discriminate between a left and right sided origin of activation. In all ΔAT measurements, a positive value indicates that the RV MAP was activated earlier than the LV MAP.

Unipolar electrogram recordings were analyzed with the same custom-written Matlab software. ARI was calculated from the minimum dV/dt of the QRS complex to the maximum dV/dt of the T wave of the unipolar electrogram, independent of T wave morphology, because this correlates most closely with MAPD.[19] Signals were excluded if signal quality was considered insufficient (e.g. due to an unstable isoelectric line or presumed loss of contact with the endocardium). Activation times for extracellular electrograms were defined as interval from pacing stimulus artefact to the modulus of the bipolar electrogram, or minimum dV/dt of the unipolar electrogram. Activation times were measured in both NavX and Prucka electrophysiology systems (R^2=0.93; average was used), while activation times of needle electrode recordings were determined using the Matlab software. Repolarization time was calculated by adding activation time and ARI. Far field

atrial deflections were discernible in the vast majority of electrogram recordings and resulted in outliers in most of the measurements, due to AV-dyssynchrony. Therefore the following filtering was applied: if the absolute difference between the ARIs of two consecutive beats exceeded three times the interquartile range of differences of all recorded samples (from baseline to first ectopic beat after dofetilide), these values were excluded for calculation of ARI and STV_{ARI}.

All measurements after dofetilide were performed before the induction of the first ectopic beat. During needle mapping the activation time of all electrograms of a typical ectopic beat and TdP episode were analyzed to determine the area of earliest activation. The neighboring electrograms were analyzed to determine local spatial dispersion. The selection of the neighboring electrograms is visualized in *Figure 1*.

Statistical analysis

Electrophysiological parameters of *Table 1* were compared with one-way repeated measures ANOVA. Post hoc multiple comparisons were performed with paired Student's *t*-tests with Bonferroni correction versus chronic RVA baseline. Relative differences in mRNA expression levels (RT-qPCR) were compared with unpaired Student's *t*-tests. If requirements of normal distributions were violated, logarithmic transformation was performed or an equivalent nonparametric test was used. Two-way repeated measures ANOVA was used for analysis of the epicardial activation times. The regression lines were calculated by simple linear regression and the adjusted R^2 values reported. A P value smaller than 0.05 was considered significant.

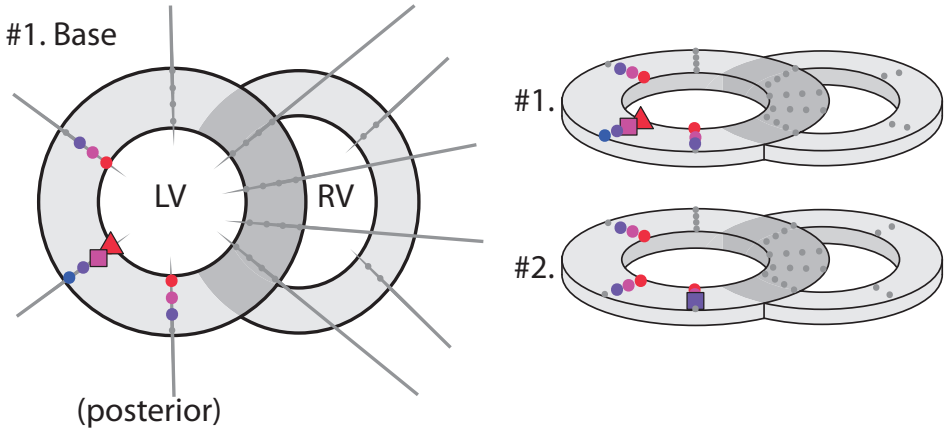


Figure 1. Schematic overview of needle placement.

Needles were inserted at six evenly distributed levels (#1-6, from base to apex). At each level up to ten needles were inserted, each with four or two electrode terminals (grey dots/symbols). The left panel shows the most basally located section. The symbols and colors are added to indicate the commonly observed area of induction of the ectopic beat after dofetilide (For explanation of symbols see Figure 4D).

RESULTS

Effects of RVA pacing and bradycardia on activation and repolarization

RVA pacing acutely after AV-block resulted in a wide QRS of 113±15 ms, with an earlier activation time of RV than LV MAP (positive ΔAT in *Table 1*) and this sequence was not influenced by the remodeling period.

Global repolarization duration, as evaluated by QT and JT, showed a small, but clear trend towards an increase after chronic, bradycardic RVA pacing (+11%, P=0.056 and +15%, P=0.095, respectively; *Table 1*). However, the remodeling due to chronic RVA pacing was neither associated with increase in STV_{LV}, (P=1.00) nor LV MAPD (+5%; P=0.54) nor RV MAPD (+11%, P=0.16). Albeit not significant, the larger increase of RV than LV MAPD prevented an increase of ΔMAPD. Also $T_{peak\text{-}end}$ was not increased by the remodeling period (+16%, P=0.45).

Effects of dofetilide after chronic RVA pacing with bradycardia

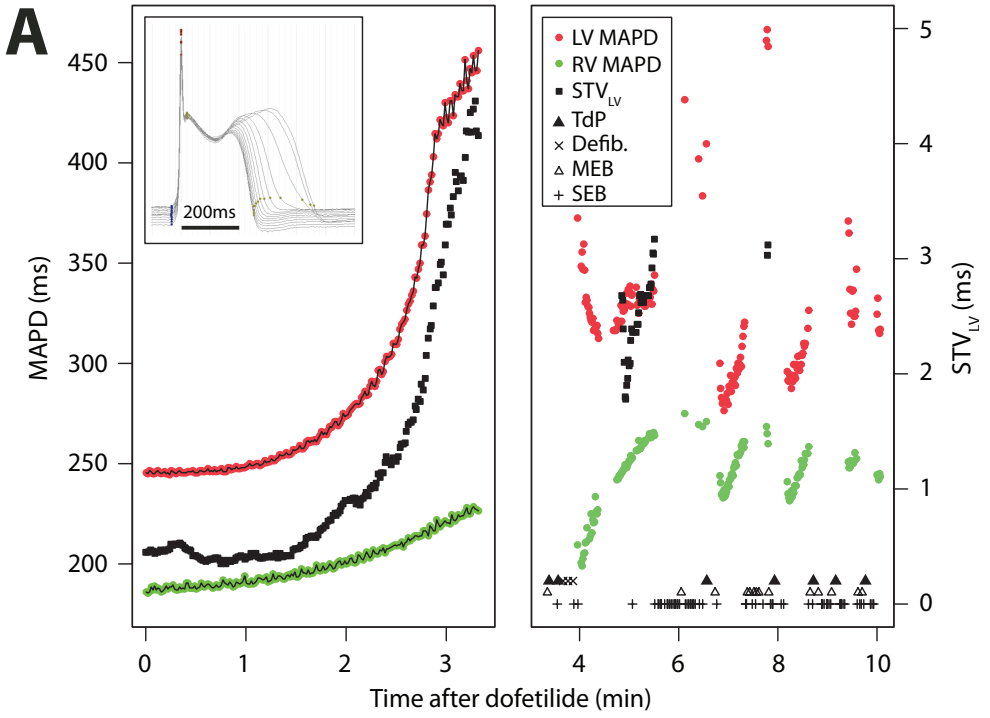
After dofetilide repetitive TdP episodes were induced in six out of eight dogs (75%). Dofetilide did not influence the activation pattern: the RV MAP remained earlier activated than the LV MAP, without change in QRS. LV repolarization however was severely prolonged (MAPD: +59%; P<0.001) and to a much larger extent than RV MAPD, resulting in a large increase of ΔMAPD (to 89±81ms; *Table 1*, right column). $T_{peak\text{-}end}$ was increased significantly (+82%,

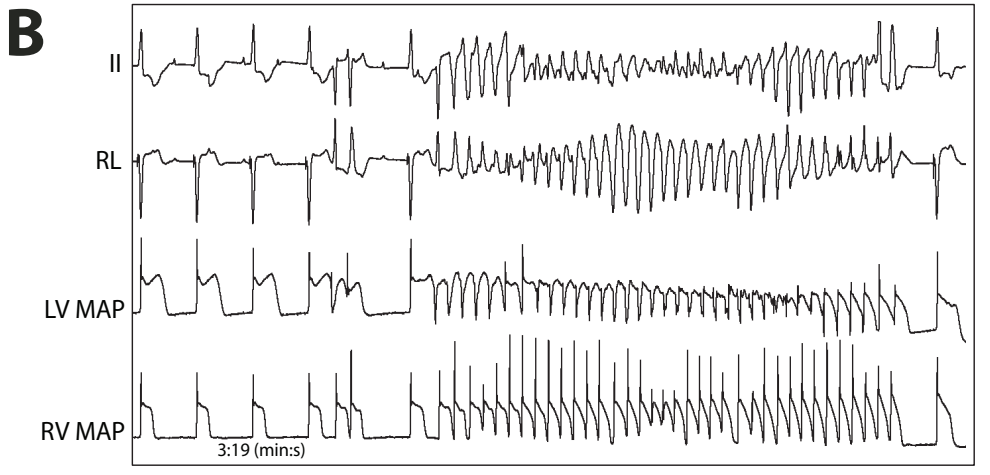
Table 1. Electrophysiological effects of chronic, bradycardic RVA pacing and the effect of dofetilide after remodeling

	RVA 0	RVA 3	RVA 3 + Dof.	P values	
				3 vs. 0	Dof. vs. 3
QRS	113 ± 15	118 ± 12	121 ± 11	NS	NS
ΔAT	25 ± 14	28 ± 14	28 ± 15	1.00	1.00
QT	343 ± 23	381 ± 29	545 ± 63 ***	0.056	<0.001
JT	213 ± 20	245 ± 23	406 ± 65 ***	0.095	<0.001
$T_{peak\text{-}end}$	90 ± 14	100 ± 10	213 ± 60 ***	0.45	<0.001
LV MAPD	245 ± 14	256 ± 23	411 ± 70 ***	0.54	<0.001
RV MAPD	217 ± 14	242 ± 38	322 ± 87 *	0.16	0.013
ΔMAPD	27 ± 10	13 ± 30	89 ± 81 *	0.54	0.012
STV_{LV}	0.8 ± 0.4	0.7 ± 0.3	3.7 ± 2.9 **	1.00	0.004
STV_{RV}	1.1 ± 0.3	1.4 ± 1.0	2.3 ± 1.6	0.98	0.073

ΔAT, activation time of left ventricular (LV) monophasic action potential (MAP) minus activation time of right ventricular (RV) MAP; MAPD, MAP duration; ΔMAPD, difference between LV and RV MAPD; NS, ANOVA not significant, no post hoc test performed; STV_{LV}, short-term variability of LV MAPD; STV_{RV}, short-term variability of RV MAPD; 3 vs. 0, RVA 3 weeks versus RVA 0 weeks; Dof. vs. 3, dofetilide versus baseline (during RVA 3 weeks). All values are mean ± SD, in ms. */**/***, P<0.05/<0.01/<0.001 vs. RVA 3.

P<0.001), just like STV_{LV}: a 4.5 times increase (P=0.004). Individual examples of the increase of LV MAPD and RV MAPD, the beat-to-beat variability and the occurrence of a self-terminating TdP are shown in *Figure 2*. Moreover, their dynamic behavior in-between TdPs is depicted in the right panel of *Figure 2A*.





mRNA expression

In *Figure 3*, the relative expression of a number of ion channels and calcium-handling genes participating in the excitation contraction process has been listed. A significant downregulation was present in a number of genes (CASQ2, KCNIP2, KCNJ11, NCX1) in the RVA paced AV-block dog compared to unremodeled control animals with normally conducted sinus rhythm.

Regional differences in electrophysiological remodeling and arrhythmogenesis

The mean ΔAT_{EB} was 25±25 ms (median 15 ms, range 1 to 34 ms) and the mean ΔAT_{TdP} was 44±45 ms (median 44 ms, range -17 to 106 ms), indicating a predominant LV origin of both the first ectopic beats and the beats during onset of TdP.

In the two additional dogs, serial analysis before and after remodeling revealed that the LV ARI values increased from 243±9 ms (n=46) to 264±13 ms (n=50) in dog #1, whereas no prolongation was observed in dog #2: 244±11 ms (n=49) versus 238±13 ms (n=44). These LV ARI data were in agreement with the measured LV MAPD values, which were on average slightly prolonged, but not changed significantly (*Table 1*). Also mean RV ARI samples were in agreement with the mapping data, because only a small increase was present in both dogs: in dog #1 from 209±8 ms (n=43) to 226±26 ms (n=36) and in dog #2 from 210±8 (n=37) to 221±20 ms (n=46). The mean activation times before and after remodeling were roughly similar in dog #1 (LV: 62±11 and 64±8 ms; RV: 40±12 and 33±13 ms) and dog #2 (LV: 60±13 and 68±11 ms; RV: 33±12 and 40±14 ms). After remodeling a more negative relationship between activation time and ARI was observed in both dogs (*Figure 4A; Table 2*), both for the RV and LV ARIs. This was mainly caused by ARI prolongation in the earlier activated LV and RV regions.

Figure 2 (left page). Individual example showing the dofetilide-induced changes in monophasic action potential duration (MAPD).

A: Increase in left and right ventricular (LV and RV) MAPD, during (left panel) and after (right panel) infusion of dofetilide. In this dog the onset of ectopic activity (at 3:20s in panel B) was immediately followed by Torsade de Pointes (TdP). As a consequence infusion of dofetilide was then aborted. Visible is the very large increase of the LV MAPD relative to RV MAPD, which resulted in an increase of spatial (interventricular) dispersion of repolarization just before onset of arrhythmogenesis. The average morphology of the LV MAP during the infusion of dofetilide is shown in the inset in panel A. LV short-term variability (STV_{LV}) also increased during infusion and was largest just before onset of arrhythmogenesis. The right panel shows the dynamic behavior of MAPD and STV_{LV} during periods free of arrhythmias. Arrhythmogenesis over time is shown at the bottom of the panel. Episodes of TdP resulted in temporary shortening of the absolute MAPD values, while TdP episodes appeared to occur preferentially when LV MAPD was longest. STV_{LV} could only be estimated at a few time points, because the parameter required 30 beats.

Abbreviations: Defib, defibrillation; MEB, run of multiple (3-5) ectopic beats; SEB, single ectopic beat.

B: Surface ECG (lead II and the precordial lead RL) and the LV and RV MAP showing the induction of the first ectopic beat and TdP episode. This TdP was followed by only a single regular beat, without ectopic beat (shown). Then a second episode of TdP occurred that was terminated only after three electrical cardioversions (not shown).

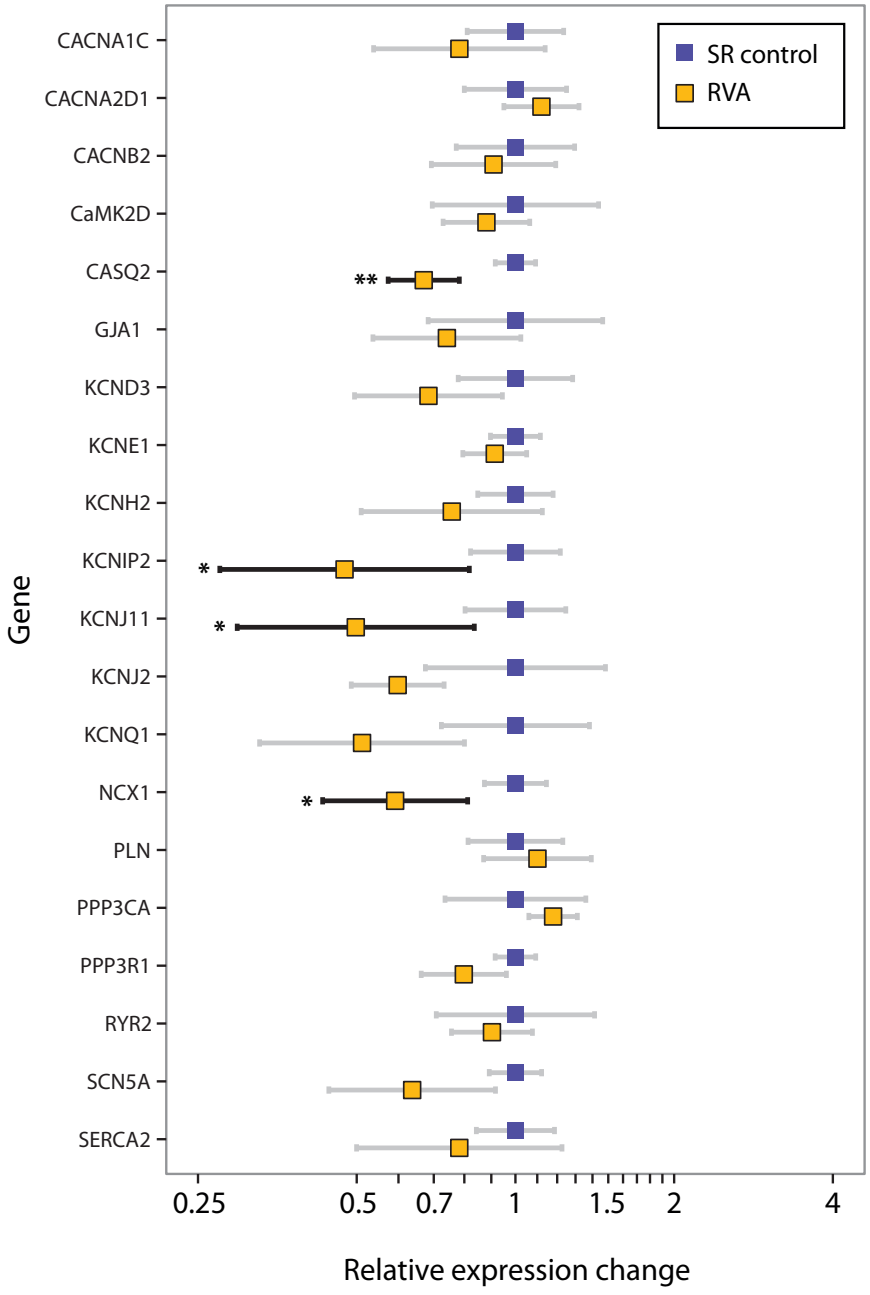


Figure 3.

Effect of ventricular remodeling due to chronic bradycardic RVA pacing on mRNA expression of a number of ion channel encoding genes, compared to unremodeled, normal sinus rhythm (SR) controls. Samples from the left ventricular antero-lateral free wall were compared.

*/**, P<0.05/<0.01 vs SR.

The LV intramural data were similar to the endocardial data: a clear relationship between ARI and activation time was present, independent of the intramural recording site (*Figure 4B*). The mean ARI of LV was 285±20 ms, which was slightly longer than during the endocardial measurements.

Administration of dofetilide resulted in prolongation of ARIs. The ARIs after dofetilide were closely correlated with ARI at baseline: adjusted R^2=0.81, P<0.001, slope 1.6 (95%-CI: 1.4 – 1.8). Due to the more pronounced increase of ARIs in the earlier activated areas, the slope of the linear fit of the ARI-AT relationship became more negative (*Table 2* and *Figure 4B*). Moreover, especially in the earlier activated regions, spatial heterogeneity of repolarization times was visually increased (*Figure 4D*). In this dog repetitive TdP episodes were not induced after dofetilide administration, but runs of ectopic beats and a single (borderline) TdP episode were observed. Analysis of the activation time of a 'typical' ectopic beat and of the first beat of the TdP episode, confirmed the LV origin (posterolateral base). Repolarization times of neighboring needle electrodes (*Figure 1*) revealed that a large difference in spatial dispersion was involved (*Figure 4D*). The other four beats of the TdP episode originated from various LV sites and local maximal dispersion of repolarization times was 123, 153, 133 and 82 ms respectively (based on analysis of neighboring electrogram signals). Three out of four ectopic beats were initiated before repolarization of the latest repolarizing neighbor electrogram.

STV_{ARI} was determined at different transmural sites to investigate whether relevant heterogeneity was present. The mean STV_{ARI} was 1.1±0.7 (median [interquartile range]: 1.0 [0.6-1.4]): which is low and roughly similar to the values of STV of the LV MAPD in *Table 1*. STV_{ARI} showed large heterogeneity but was correlated with activation time as well (*Figure 5A*). Values higher than 2 were almost exclusively observed in the earlier activated areas. After dofetilide we did observe a large increase of STV_{ARI} (mean 3.3±2, median 2.6 [1.8-4.8]), which was most pronounced in the earlier activated regions, but heterogeneity of STV_{ARI} was also increased in the later activated regions (*Figure 4B*).

Termination

Following excision of the heart, the RVA lead position was confirmed visually. Heart-to-body-weight ratio of the eight dogs that were tested for susceptibility was (0.0102±0.0006) and the heart weight was 228±39 g. No signs of heart failure (including ascites, weight gain or inactivity) were observed in these dogs. Lung weight was 205±32 g.

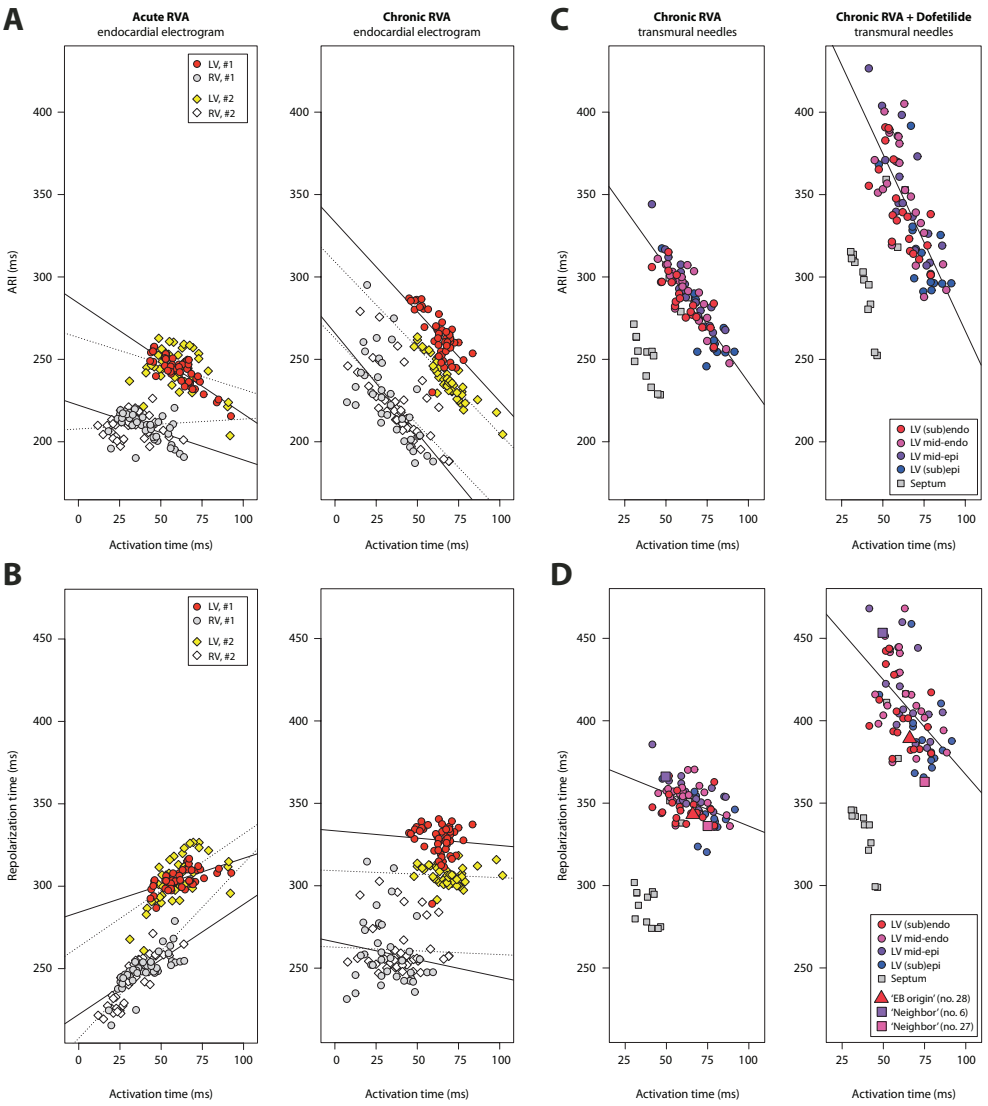


Figure 4. Activation time (AT) dependent intraventricular heterogeneity of repolarization.

A: LV and RV activation recovery interval (ARI) versus AT, before (left) and after (right) remodeling due to chronic bradycardic RVA pacing. As expected, LV ARI was longer than RV ARI. Only in dog #1 the remodeling was associated with a small overall prolongation of LV ARI, while in both dogs the slopes of the relationship with AT became more negative (*Table 2*).

B: The remodeling was associated with a more horizontal slope of the relation between repolarization time (=ARI + AT) and AT, indicating that the net 'early to late' gradient of repolarization time was eliminated by remodeling.

C/D: Intramural ARI recordings during open thorax needle mapping (dog #1). The septal electrodes were excluded in the calculations of the simple linear regression lines, to insure against inclusion of RV measurements.

C: A negative relation between AT and ARI (observed in A) was confirmed in the analysis of transmural data during

needle mapping, while no relevant AT independent transmural differences in ARI were observed. After dofetilide (right panel), the AT-ARI slope was decreased further, because repolarization was prolonged preferentially in earlier activated regions.

D: The almost horizontal slope of the AT-ARI relation at baseline (left panel) became more negative after administration of dofetilide (right panel). However, not only the 'early to late' repolarization gradient was increased: the heterogeneity of repolarization time within the early-activated LV region was also increased considerably. Ectopic beats were induced in an area with very high spatial dispersion of repolarization moments. This is illustrated by showing the neighbor ARI with longest and shortest repolarization time (larger symbols).

Table 2. Correlation between activation time and repolarization in the left ventricle

	Endocardial mapping		Transmural mapping	
	0 weeks	4 weeks		
	Baseline			Dofetilide
AT-ARI				
Slope	-0.7	-1.1	-1.4	-2.2
	(-0.3)	(-1.0)		
95%-CI	-0.8 – -0.5	-1.4 – -0.8	-1.6 – -1.2	-2.6 – -1.7
	(-0.6 – -0.1)	(-1.2 – -0.9)		
R^2	0.69	0.47	0.75	0.56
	(0.10)	(0.82)		
P value	<0.001	<0.001	<0.001	<0.001
	(0.014)	(<0.001)		
AT-RT				
Slope	0.3	-0.1	-0.4	-1.2
	(0.7)	(-0.0)		
95%-CI	0.2 – 0.5	-0.4 – 0.2	-0.6 – -0.2	-1.6 – -0.7
	(0.4 – 0.9)	(-0.2 – 0.1)		
R^2	0.33	-0.01	0.19	0.26
	(0.38)	(-0.02)		
P value	<0.001	0.59	<0.001	<0.001
	(<0.001)	(0.58)		

AT, activation time; ARI, activation recovery interval; RT, repolarization time; 95%-CI, 95% confidence interval of the slope of the linear regression line. Values from dog #1 (black) and dog #2 (grey), from *Figure 4*.

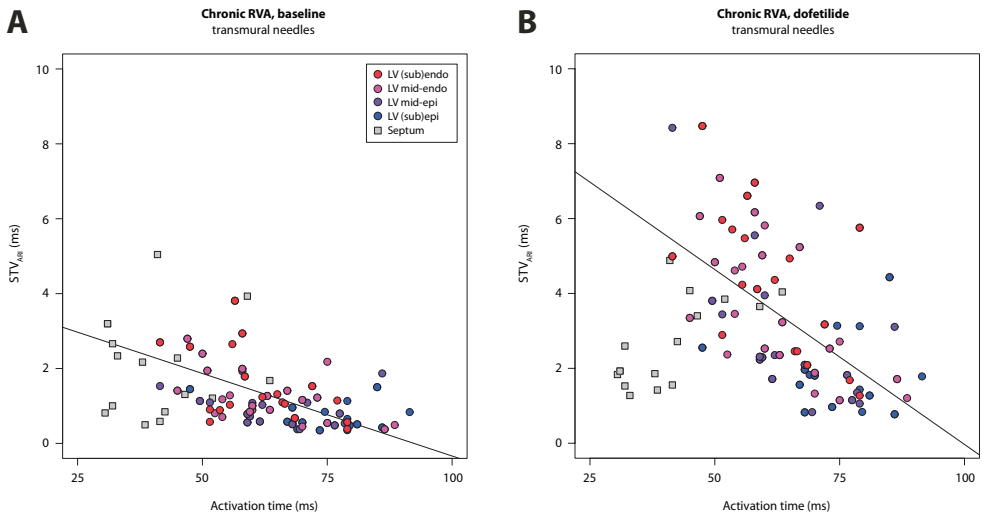


Figure 5

Intraventricular heterogeneity of short-term-variability of left ventricular transmural activation recovery intervals (STV_{ARI}) and the relation with activation time, at baseline (A) and after dofetilide (B).

At baseline, the median STV_{ARI} was 0.98 ms, but considerable heterogeneity was present. This could only partly be explained by activation time: R^2=0.21, P<0.001, slope of the linear regression line (95% confidence interval): -0.028 (-0.040 – -0.016). Due to this relationship, high values of >2 ms were almost exclusively measured in the earlier activated regions. After dofetilide administration, STV_{ARI} was increased considerably (median 2.6 ms) and the slope became more negative: -0.097 (-0.13 – -0.067), R^2=0.35, P<0.001.

DISCUSSION

Arrhythmogenesis of TdP and the relation with electrophysiological remodeling in the CAVB dog model

In general, cardiac arrhythmias are based on either abnormal impulse initiation or abnormal impulse conduction, or a combination.[20] TdP is mostly assumed to be caused by triggered activity related to early (EAD) or delayed (DAD) afterdepolarizations, but especially the mechanism of perpetuation is still matter of debate. This may be based on re-entry or focal activity.[21] In the canine CAVB model, both mechanisms have been demonstrated in mapping studies with transmural needle electrodes.[16,22,23] Overall these data suggest that focal activity is most important, especially for initiation and during the initial part of TdP arrhythmias, whereas circus movement seems to occur in a later phase of the arrhythmia. These studies also showed that focal activity is induced mostly in the LV; the first beat typically occurs at a subendocardial site, whereas subsequent beats could originate also near the epicardium.

TdP is typically observed in the setting of a prolonged QT interval. (Heterogeneous) prolongation of repolarization is a hallmark of ventricular electrical remodeling in several cardiac diseases

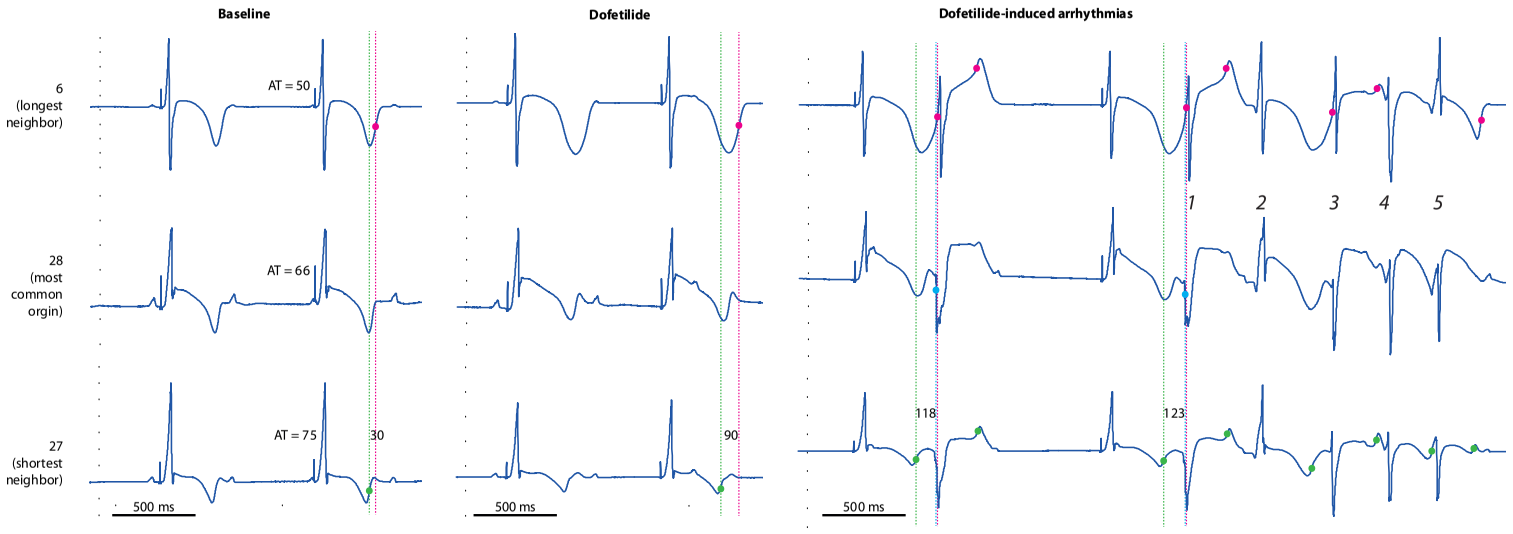


Figure 6. Example of dofetilide-induced ectopic beats and the relation with spatial dispersion of repolarization.

During the typical ectopic beat after dofetilide, electrogram signal no. 28 (middle tracing, right panel) had the shortest activation time (blue dots/lines). Analysis of the neighboring electrograms (see also *Figure 1 and 4*) showed that, of the stimlated beats, no. 6 (upper tracing) repolarized latest (red dots), while no. 27 repolarized earliest (green dots). This resulted in a spatial dispersion of repolarization times of 90 ms after dofetilide (interval between the green and red line in the middle panel), which was increased further when ectopic beats and TdP (an episode of 5 beats, numbered in the figure) were induced (right panel). Spatial dispersion was already present at baseline (left): in spite of the fact that no. 27 was activated 25 ms later than no. 6 it repolarized 30 ms earlier.

(especially heart failure) and associated with an increased susceptibility to arrhythmias.[24-26] In the CAVB model, repolarization prolongation is present and linked to changes in ventricular ionic currents, including downregulation of delayed rectifier potassium currents.[27] The prolongation of repolarization is about 20-30%, measured during idioventricular rhythm at CAVB, compared to acutely after onset of AV-block.[13,28,29] The large increase of QT is preserved when temporary RVA pacing is performed at a rate of 60/min: an increase of 24% of the mean QT was still observed in CAVB dogs.[13]

Electrical remodeling due to chronic idioventricular rhythm in AV-block dogs is spatially not homogeneously distributed: at acute AV-block the RV MAPD is slightly shorter than the LV MAPD, a pre-existent difference of the electrophysiological characteristics. Ventricular remodeling results in a larger increase of LV than RV MAPD (roughly +30% and +20%, respectively), enhancing this pre-existent interventricular difference in repolarization duration (ΔMAPD increases). This is in agreement with a more dominant role of the LV for arrhythmogenesis. I_{Kr}-block further prolongs repolarization and ΔMAPD.[12,13,28,29] A similar pattern is observed in STV_{LV}: the lowest value is observed at acute AV-block (roughly 1 ms), remodeling due to CAVB results in roughly doubling of STV_{LV} and STV_{LV} is doubled again by dofetilide administration in CAVB dogs.[28]

Apparent reduction of electrophysiological remodeling by chronic RVA pacing

The hypothesis of the present study, that RVA pacing would attenuate proarrhythmic LV remodeling compared to normal AV-block dogs was not only based on the previously reported attenuated proarrhythmic electrical remodeling in high-septally paced AV-block dogs,[13] but also on observations in two AV-block dogs that required chronic pacing due to a too slow idioventricular rhythm. These dogs that had been paced chronically from the RV (apex and free wall) in VVI mode at a heart rate of 40-45 beats per minute were not susceptible to dofetilide-induced TdP.

In the present study, chronic RVA pacing was not associated with a significant increase of LV MAPD and ΔMAPD, while QT increased by a relatively modest 11%, which was statistically only a trend (P=0.06). In addition, STV_{LV}, which is considered a better predictor of TdP risk than QT in this model,[28] was not influenced by remodeling and neither was $T_{peak\text{-}end}$.

However, these dogs showed a high incidence of TdP (6/8), indicating that proarrhythmic remodeling was not prevented. Moreover, dofetilide administration seemed to 'unmask' the electrical remodeling: electrophysiological parameters, including LV MAPD and STV_{LV}, increased significantly.

The latter finding suggests that the proarrhythmic remodeling was disguised by other factors. It is known that repolarization duration is not closely related to arrhythmic risk. Due to the complex interplay between depolarizing and repolarizing currents and the electrical coupling between cells, action potentials might appear quite similar, while possessing different proarrhythmic potential.[30] Moreover, the ability to induce EADs and spatial dispersion (upon I_{Kr} block) are most likely more important than repolarization prolongation per se.[31-33] The limitation of QT to

predict drug-induced TdP has been an important reason for the use of STV_{LV}, which performed better in many studies.[18,28]

RT-qPCR analysis indicated altered RNA expression of specific ion channel and calcium-handling genes in the LV free wall of RVA paced CAVB dogs as compared to normal sinus rhythm. In particular, the RNA levels for KCNIP2 (contributing to I_{to1}), KCNJ11 (underlying $I_{K,ATP}$), NCX1 (N^{a+}-Ca^{2+} exchanger) and CASQ2 (calsequestrin 2) were significantly reduced. But also other ion channel genes such as KCNQ1 (determining I_{Ks}) and SCN5A (underlying I_{Na}) appeared to be less well expressed. These results suggest changes in determinants of cardiac electrical activity, thereby potentially contributing to the proarrhythmic state of the LV in RVA paced CAVB hearts. Extrapolation of the observed alterations in RNA expression to the mechanism of proarrhythmia proves less obvious. For that, not only the protein levels and function of the respective molecules but also the multiprotein complexes in which they participate (often consisting of auxiliary, structural and regulatory proteins) need to be considered. Nevertheless, these molecular results represent a first starting point for an in-depth study towards such mechanism in this model.

Intraventricular heterogeneity in ventricular electrophysiological remodeling: conflicting data in the literature

To exclude the presence of significant heterogeneity of repolarization and to further explore heterogeneity of STV_{LV}, additional mapping experiments were performed. A dyssynchronous LV activation pattern results in mechanical dyssynchrony and is linked to heterogeneous electrical remodeling, but conflicting data exist: some studies suggest that remodeling is most pronounced in the latest activated region related to the increased strain in that region (early systolic prestretching occurs in latest activated areas), whereas others suggest that the early activated regions remodel most. Jeyaraj and coworkers have emphasized the importance of the latest activated region. They have shown that four weeks of epicardial LV pacing resulted in progressive development of T wave memory in dogs.[34] Multisegment transmural optical mapping was performed of myocardial wedges from the late activated, high strain area and compared to segments from early activated myocardium. Longest action potential durations were found in the latest activated area, independent of pacing site. No hypertrophy was present after four weeks.

Spragg[35] found a more pronounced increase of action potential duration in early activated regions compared to late activated regions, in myocardial wedges isolated from dogs with chronic LBBB, but no significant differences versus a control group were present and no relation with increased arrhythmogenesis was found.

In another study, conducted at the same institution, the importance of the late activated region was stressed.[36] Heart failure was induced in dogs by LBBB combined with (atrial) pacing-induced tachycardia. This was either continued for six weeks, or continued for three weeks followed by three weeks of cardiac resynchronization therapy to restore synchrony of activation, while maintaining tachycardia. Isolated cardiomyocytes were analyzed to measure action potential duration: the remodeling was associated with more pronounced increase of action potential duration in

myocytes isolated from the lateral wall (late activated) than the anterior wall (early activated), but a similar EAD inducibility was seen. Cardiac resynchronization partially restored the action potential duration, especially in the lateral cells.

'Cardiac memory' refers to changes of the T wave due to altered ventricular activation that are visible for some time when the original activation is restored.[37] Costard-Jackle[38] studied short-term cardiac memory by epicardial LV pacing in Langendorff-perfused rabbit hearts. Pacing resulted in a slow decrease of the slope of the relation between action potential duration and activation time, from -0.2 (at 5 min) to -0.7, two hours after onset of pacing, whereas in atrially paced control hearts the slope remained slightly lower than -1. Arrhythmogenesis was not studied and only epicardial action potentials were analyzed in this study.

We found that chronic bradycardic RVA pacing was associated with significant inter- and intraventricular heterogeneity of repolarization: the slope of the relation between activation time and ARI was reduced from about -0.5 to about -1.0 after remodeling (n=2). These data are in agreement with the effects of short-term memory.[38]

TdP has often been associated with spatial dispersion of repolarization, but conflicting data exist in the literature. Especially, the importance of transmural differences in repolarization caused by M cells is controversial. In a review by Janse[39] it was discussed that, although in wedge preparations and myocardial slices M cells can be detected, these differences are attenuated due to electrotonic coupling of cells in vivo.

In our in vivo study, we did not observe a remarkable transmural difference in ARI and repolarization times, other than caused by differences in activation time (based on the plots in *Figure 4*). However, heterogeneity of repolarization times was present and accentuated by dofetilide. The observation that the ectopic beats were induced at a region with large spatial dispersion of repolarization (but also activation), may suggest that the induction was caused by an electrotonic interaction, while conduction might have been facilitated by shorter ARIs, mostly present in the late activated regions. Based on our findings we cannot assign either the early or the late activated area of the LV as origin of proarrhythmia.

The absence of significant QT prolongation is in agreement with the observation in the mapping experiments, which demonstrated preferential prolongation of repolarization in the earlier-activated regions. At baseline these regions repolarized earlier than the latest activated regions (*Figure 4B*). As a consequence, repolarization prolongation in these areas may have been (partly) masked by the later activated regions, during the analysis of the QT interval before and after remodeling.

Finally a remark on STV of ARI: we observed substantial heterogeneity, which was only partly related to activation time. This suggests that a single measurement at one site may be insufficient for reliable risk prediction of TdP in an individual.

Implications for the CAVB dog model

Due to the importance of activation pattern for electrophysiological remodeling in the CAVB dog, the model may be better standardized if the uncontrolled idioventricular rhythm is replaced

with a constant activation pattern using chronic bradycardic pacing. To reduce disguising of electrophysiological remodeling, LV rather than RV pacing may be preferable.

Limitations

In this study each dog served as its own control (serial experiments), but the acute proarrhythmic effect of RVA pacing was not studied. Therefore, we cannot exclude that an acute proarrhythmic effect of RVA pacing has contributed to the observed proarrhythmic effect of chronic bradycardic RVA pacing. Although the proarrhythmic susceptibility appeared to be similar to CAVB dogs with chronic idioventricular rhythm, due to the small sample size we cannot exclude that the arrhythmia susceptibility was attenuated (like in chronic bradycardic high-septal paced dogs[13]). Serial mapping of repolarization was performed in only two dogs and therefore further research is required to validate these findings. Based on our data we can neither confirm nor exclude that EADs were involved. Functional and structural remodeling were not determined in this study, except for heart-to body weight ratio at sacrifice, which was low in comparison to reported values in CAVB and high-septal paced dogs;[13] but also compared to matched CAVB dogs (n=7; matching based on duration of AV-block and gender), which showed a heart-to-body weight ratio of 0.0125±0.0024).

Conclusions

Chronic bradycardic RVA pacing in dogs enhanced susceptibility to dofetilide-induced arrhythmias considerably, but this was not associated with significant changes in commonly used parameters to quantify electrical remodeling, including LV MAPD, STV and $T_{peak\text{-}end}$. However, dofetilide caused large increases of these parameters, apparently unmasking electrical remodeling. Moreover, pronounced prolongation of ARIs was detected in the early activated LV regions. The LV heterogeneity of repolarization times was amplified by dofetilide and induction of ectopic beats was associated with large local LV spatial dispersion.

Acknowledgements

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SUPPLEMENT

Supplemental Table 1.

Target gene	Gene expression ID
SCN5A	Cf02625032_m1
KCND3	Cf02698011_m1
KCNIP2	Cf02630856_m1
KCNQ1	Cf02690510_m1
KCNE1	Cf02690512_g1
KCNH2	Cf02624782_m1
KCNJ2	Cf03022918_s1
KCNJ11	Cf02625250_s1
GJA1	Cf02690400_g1
CACNA1C	Cf02625954_m1
HPRT1	Cf02626256_m1
CACNA2D1	Cf02659475_m1
CACNB2	Cf02681339_m1
CACNA1G	Cf02723988_m1
CACNA1H	Cf02625946_m1
RYR2	Cf02624383_m1
PLN	Cf02625405_m1
PPP3R1	Cf02660925_m1
PPP3CA	Cf02684302_m1
SERCA2A	Cf02695995_m1
CAMK2D	Cf02660408_m1
CASQ2	Cf02695399_m1
NCX1	Cf02724810_m1
RPLP1	Cf02734960_g1

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Chapter 6

Novel parameters to improve quantification of arrhythmogenesis and risk of Torsade de Pointes using a dofetilide challenge in anesthetized dogs with complete AV-block

Thom R.G. Stams[1], Peter Oosterhoff[1], Stephan K.G. Winckels[1], Avram Oros[1], Rosanne Varkevisser[1], Roel van der Nagel[1], Jet D.M. Beekman[1], Marc A. Vos[1]

[1] Department of Medical Physiology, Division Heart & Lungs, University Medical Center Utrecht, Utrecht, The Netherlands

[2] ICIN-Netherlands Heart Institute, Utrecht, The Netherlands

[3] Department of Cardiology, St. Antonius Hospital, Nieuwegein, The Netherlands

Submitted

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ABSTRACT

Background: in the canine, complete AV-block model, evaluation for proarrhythmia using inducibility of Torsade de Pointes (TdP) as primary outcome can be cumbersome, because inducibility is dichotomous and requires ≥3 episodes. We aimed to improve quantification and prediction of arrhythmias using novel parameters arrhythmia score (AS) and T25.

Methods: Experiments from four groups of AV-block dogs under general anesthesia were analyzed: acute AV-block (aAVB, n=13) and dogs with remodeled hearts due to chronic bradycardia but different chronic activation pattern: spontaneous idioventricular rhythm (IVR; n=19), high-septal pacing (HSP; n=10) and right ventricular apex pacing (RVA; n=8). Number of single and multiple ectopic beats, TdP duration and number of cardioversions were combined in one arrhythmia score (range 1-100), using the three most severe proarrhythmic events after dofetilide. T25 was defined as time required to obtain a 25ms-increase of LV MAPD after dofetilide (0.025mg/kg/5min).

Results: 'Predicting' inducibility after pooling all data (n=50), yielded areas under the receiver operating characteristics curves of 1.00 for AS, and 0.79 for T25. AS was lowest in aAVB and HSP and highest in IVR ($P<0.001$ vs. aAVB and $P<0.05$ vs. HSP). In aAVB dogs AS was significantly increased by dofetilide, while inducibility remained 0%. T25 in aAVB, HSP, RVA and IVR was 2.1±0.4, 1.9±0.4, 1.5±0.3 ($P<0.01$ vs. aAVB) and 1.4±0.4min ($P<0.001$ vs. aAVB; $P<0.01$ vs. HSP), respectively.

Conclusions: In anesthetized AV-block dogs, AS and T25 may improve quantification and prediction of proarrhythmia.

Keywords: Ventricular arrhythmias; Torsade de Pointes; Acquired long QT syndrome; Electrophysiology; Cardiac remodeling; Dofetilide

INTRODUCTION

The anesthetized canine with chronic, complete AV-block has been used extensively as a model to study drug-induced Torsade de Pointes arrhythmias (TdP). Application of the specific blocker of the delayed rectifier potassium current (I_{Kr}) dofetilide (reference compound) results in a high incidence of repetitive TdP episodes (about 75%).

Creation of AV-block leads to (1) bradycardia, (2) AV-dyssynchrony, and (3) abnormal ventricular activation, and acutely reduces cardiac output. In time, cardiac output is almost completely restored due to the occurrence of ventricular remodeling.[1] The proarrhythmic remodeling is not only dependent on chronic bradycardia, but can be modulated by altering ventricular activation during the remodeling period. AV-block dogs paced chronically from the high septum at lowest captured rate (HSP), showed less severe TdP episodes and a lower incidence than unpaced, chronic AV-block dogs with remodeling due to chronic, bradycardic idioventricular rhythm (IVR): 4/9 (44%) vs. 7/9 (78%), P=0.17.[2] This trend towards TdP reduction has been explained by the more physiological ventricular activation pattern. The relevance of abnormal ventricular activation was recently confirmed in another study in which AV-block dogs were paced from the right ventricular apex (RVA), a hemodynamically less favorable position, with chronic bradycardia due to pacing at the lowest captured rate: these dogs showed an incidence of 6/8 (75%) [*Chapter 5*].

Quantification of arrhythmia severity using a dichotomous parameter like incidence can be cumbersome. In studies in dogs, typically small sample sizes are used. As a consequence, even at a statistical power level of 0.8, large effect sizes are required. Winckels et al. found an about 50% reduction of TdP incidence, but this was not significant. However, use of additional quantification methods showed that the severity of the TdP episodes was reduced.[2]

A similar problem is frequently observed in non-inducible dogs, that do show an arrhythmic response in the form of increased ectopic activity, because single and multiple ectopic beats and single episodes of TdP are not included in TdP incidence (lack of sensitivity).

Arrhythmia score was introduced with the aim to increase statistical power by combining the number of single ectopic beats, number of multiple ectopic beats, the duration of TdP episodes and the number of electrical cardioversions in a single, more continuous parameter. The primary purpose of this study was to show that arrhythmia score can at least capture the same information as TdP inducibility, while providing more detailed information on proarrhythmia.

In the chronic AV-block dog model, besides the arrhythmogenic outcome typically a number of electrophysiological parameters have been used to evaluate the proarrhythmic risk of compounds, but also to evaluate the extent of proarrhythmic electrical remodeling. These parameters include: (1) repolarization duration (QT, LV MAPD, right ventricular MAPD); (2) beat-to-beat variability of repolarization, quantified as short-term-variability (STV) of the left ventricular (LV) monophasic action potential duration (MAPD); (3) spatial dispersion of repolarization, quantified as interventricular dispersion of MAPD (ΔMAPD).[3-8] The changes in these parameters, associated with proarrhythmic remodeling due to chronic AV-block, were attenuated in a

group of AV-block dogs in which similar bradycardia, but a more physiological ventricular activation was established by chronic, high-septal pacing (HSP) at lowest captured rate.[2] This was associated with reduced TdP severity. However, an electrophysiological pattern similar to HSP was also observed in AV-block dogs with abnormal ventricular activation due to RVA pacing at lowest captured rate; these dogs showed the same high TdP incidence of 75% as dogs at IVR [see *Chapter 5*].

STV is considered to have higher predictive value for TdP than QT and ΔMAPD in this model.[1,3,4,9] Measurements of STV after dofetilide are performed during the last 30-beats window prior to the first ectopic beat.[3,6] In this study we evaluated whether the initial, absolute rise of LV MAPD by dofetilide does also predict TdP. The primary aim was to obtain a marker that is more sensitive than other parameters after dofetilide, because it is determined early after start of dofetilide, when arrhythmic risk is still low. For this purpose we used four groups of dogs, with known differences in arrhythmia susceptibility due to differences in ventricular remodeling. From lowest to highest reported arrhythmia susceptibility: unremodeled acute AV-block dogs (aAVB), HSP, RVA and regular idioventricular rhythm (IVR) dogs.

METHODS

Study design

A retrospective analysis was performed using four groups of AV-block dogs in which differences in proarrhythmic outcome have been linked to differences in ventricular remodeling: (1) aAVB (n=13); (2) chronic HSP at lowest captured rate (n=10); (3) chronic RVA at lowest captured rate (n=8); and (4) chronic, spontaneous IVR (n=19).

Experiments were selected based on: (1) the agents used for induction and maintenance of general anesthesia as described below, see 'Anesthesia and perioperative care'; (2) availability of standard 6-lead surface electrocardiogram (ECG) and LV MAP recordings of good quality, during spontaneous idioventricular rhythm or at a fixed paced heart rate (60 bpm); and (3) administration of dofetilide (0.025 mg/kg/5min i.v.) in the absence of other drugs. In total, 50 experiments were included.

Animal handling

All experiments had been approved by the Committee for Experiments on Animals of Utrecht University, the Netherlands or Maastricht University, the Netherlands and the experiments had been performed in accordance with the "European Directive for the Protection of Vertebrate Animals used for Experimental and Scientific Purpose, European Community Directive 86/609/CEE".

Anesthesia and perioperative care

After preparation, including administration of premedication (methadone 0.5 mg/kg, acepromazine 0.5 mg/kg and atropine 0.5 mg i.m.), general anesthesia was induced with sodium pentobarbital 25 mg/kg i.v. and maintained by isoflurane 1.5% or halothane 0.5-1% in O_2 and N_2O, 1:2. The dogs were endotracheally intubated and mechanically ventilated, with adjustments of tidal volume to maintain the end-tidal CO_2 concentration between 3.5 and 4.5%. AV-block had been created by radiofrequency ablation.[10] The femoral artery and vein were dissected (left or right side) and sheaths inserted to insert catheters, including monophasic action potential (MAP) catheters (Hugo Sachs Elektronik, Germany or EP Technologies, Sunnyvale, CA, USA).

Data analysis

Electrophysiological parameters were recorded with ECG-Auto (EMKA Technologies, France), Scapsys (Maastricht University, Maastricht, The Netherlands) or EP-TRACER (CardioTek, Maastricht, The Netherlands) and exported to custom software (Matlab, Natick USA) for electrophysiological measurements. MAPD was measured at 80% repolarization. Beat-to-beat variability of repolarization was quantified as short-term variability of LV MAPD (STV): the absolute difference between two consecutive LV MAPD values was calculated and divided by the square root of 2; next STV was calculated by averaging 30 values, thus using 31 consecutive beats.[4] Ectopic beats were defined as beats initiated before the end of preceding T wave on the surface ECG. To limit the influence of ectopic beats and RR variation (>10%) in the determination of STV, these beats and the two regular beats thereafter were excluded. Measurements after dofetilide were performed either before onset of arrhythmogenesis, or after complete infusion (t=5 min).

Use of arrhythmia score to quantify arrhythmia severity

Besides our regular parameter TdP-incidence (= TdP inducibility), in this study arrhythmia score was introduced, which was calculated as follows: every regular beat was scored 1 point (by default). If an ectopic beat or run of ectopic beats was initiated (all initiated within the T wave of the preceding beat), the score was increased by 1 point per ectopic beat to a maximum of 50, provided that the QRS displayed a polymorphic axis. If electrical cardioversion was applied, the score was 50, 75 or 100, depending on the number of cardioversions (1, 2 or ≥3, respectively). The rationale was that cardioversions were performed if an arrhythmia does not terminate within 10 s, corresponding to roughly ≥50 beats. The arrhythmia score was calculated by averaging the 3 highest values over a 15 min period from start of dofetilide infusion. Thus dogs that showed ≥3 TdPs ('inducible' according to the conventional dichotomous definition) have an arrhythmia score ≥6. Conversely, a value ≥6 does not necessarily indicate the presence of multiple episodes of TdP (e.g. single TdP requiring defibrillation). The scoring and an ECG example are shown in *figure 1A*.

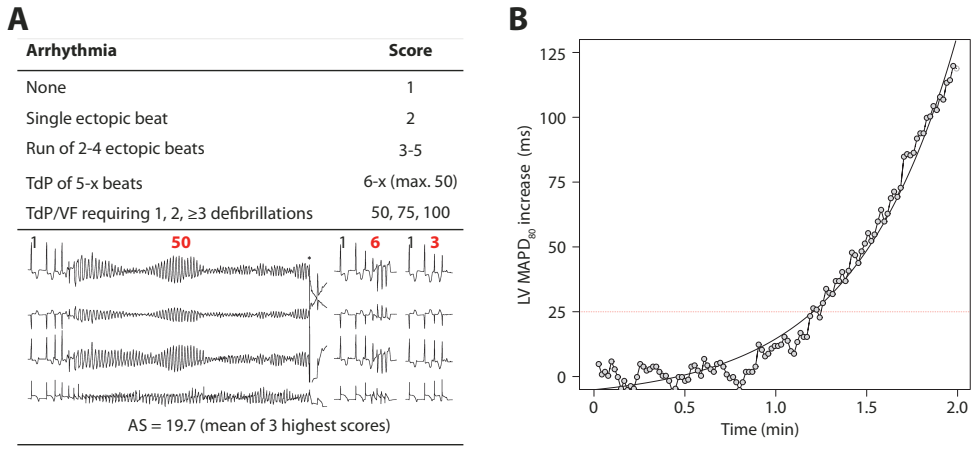


Figure 1. Calculation of arrhythmia score and T25

A: Scoring of arrhythmias, required for calculation of arrhythmia score. The ECG shows a selected example, in which two TdP episodes occurred, to further explain the calculation: the three most severe arrhythmias after dofetilide are shown (scores in red); arrhythmia score is calculated as mean of the three highest values, in this example this mean is 19.7. *, electrical cardioversion.

B: Randomly chosen example from the group of AV-block dogs with chronic idioventricular rhythm, showing the increase of left ventricular monophasic action potential duration at 80% repolarization (LV MAPD), during infusion of dofetilide (0.025 mg/kg in 5 min, start at t=0). In this dog substantial variation of LV MAPD was present at baseline. To estimate the time required to obtain a 25ms-increase of LV MAPD (T25) more precisely, an exponential fit (smooth line) was used. The threshold of 25 ms increase is marked with a red dotted line. In this example T25 (intersection of both lines) is 1.205 min.

T25 to quantify repolarization reserve

For quantification and statistical analysis of the initial, absolute increase of LV MAPD during dofetilide infusion, T25 was defined as the time from start of dofetilide infusion to obtain a 25ms-increase. This threshold was chosen because it seemed to be significantly larger than the spontaneous variation (by causes that include beat-to-beat variability of repolarization, noise and amplitude variation of the MAP signal), while it was estimated that most dogs would reach 25 ms increase of LV MAPD within 2 min after dofetilide and before induction of ectopic activity. To determine T25, the LV MAPD from t=0 s till t=120 s or until the onset of ectopic beats was used, and extended to 30 s after the estimated moment of 25ms increase, if this was not reached within 120 s, to avoid extrapolation. Because the LV MAPD showed an exponential increase with variable extent of beat-to-beat variation, an exponential fit was used to determine T25 (*Figure 1B*).

Statistical analysis

Data are expressed as mean±SD, unless stated otherwise. Inducibility was tested with Fisher's exact test, followed by all-pairwise comparisons with Fisher's-exact tests, using a Bonferroni-

adjusted P-value. One-way or two-way ANOVA with or without repeated measures (where appropriate) was used for comparison of continuous data of more than two groups. Bonferroni correction was applied for post hoc multiple comparisons. If normality assumptions were violated, logarithmic transformation was applied or ANOVA on ranks (Kruskal Wallis) was used instead, with post hoc Dunn's multiple comparisons test. Two-tailed tests were used and a P value <0.05 was considered statistically significant.

RESULTS

Comparison of arrhythmia score to TdP inducibility and predictive performance of T25

To show that arrhythmia score can at least capture the same information as TdP inducibility, we analyzed whether arrhythmia score could discriminate between inducible (repetitive TdP episodes after dofetilide) and non-inducible animals (*Figure 2A*). A receiver operating characteristic (ROC) curve was plotted and the area under the curve was 1.00 (95% confidence interval: 0.98-1.00), indicating that inducibility can be reconstructed from arrhythmia score (best cut-off value: 7.7).

T25 was a good predictor of TdP-inducibility: the ROC curve yielded an area under the curve of 0.79 (95% confidence interval: 0.65-0.93). The best cut-off value was 1.6 min (*Figure 2B*).

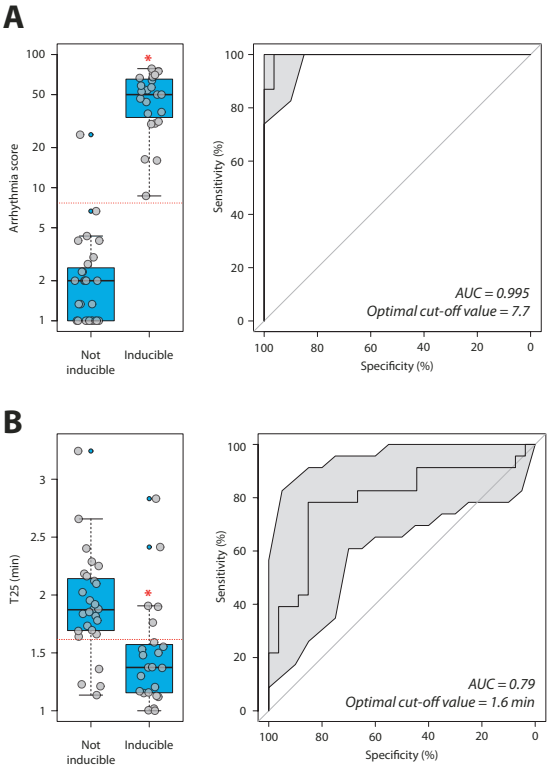


Figure 2. Accuracy of arrhythmia score and T25 to discriminate between inducible and non-inducible.

Individual data (left) and receiver-operating characteristics (ROC) curves (right) of arrhythmia score (A) or the time required to obtain a 25ms-increase of left ventricular monophasic action potential duration after start of dofetilide (T25; B).

Inducibile, three or more TdP episodes after dofetilide. *(red), P<0.001 vs. non-inducible. AUC, area under the curve. The grey shaded area represents the 95%-confidence interval of the ROC curve.

Evaluation of arrhythmia severity in the four groups using arrhythmia score

The highest arrhythmia score after dofetilide was observed in the group with chronic IVR (47 [10-65]; median [IQR]), while the arrhythmia score in RVA paced dogs was not significantly different (23 [8.2-41]). Lowest arrhythmia scores were present in aAVB and HSP (2.0 [1.0-2.3] and 1.3 [1.0-24], respectively). The latter scores were both significantly lower compared to IVR (*Figure 3*).

In aAVB, a baseline recording before start of dofetilide was available in ten dogs. A separate, paired analysis of the arrhythmia score before and after dofetilide revealed that the score was increased significantly (from 1.0 [1.0-1.3] to 1.8 [1.0-3.6], P=0.042). Typically only single ectopic beats were induced and three or more TdP episodes (required for inducibility) were never observed.

T25 in the different groups

T25 showed a similar pattern as arrhythmia score (although reversed): a significant difference between the groups was present and the post-hoc multiple comparisons versus IVR revealed that T25 was not significantly different in the RVA paced dogs (1.5±0.3 min in RVA vs. 1.4±0.4 min in IVR), but T25 was significantly longer in aAVB and HSP dogs (2.1±0.4 min and 1.9±0.4 min, respectively) (*Figure 4*).

Use of STV and MAPD to predict TdP

At baseline, no differences in STV were present between the four groups. However, after dofetilide administration, differences were present in STV between the groups (*Figure 5*). Furthermore, only after dofetilide the pattern of values correlated with the observed TdP inducibility and arrhythmia severity in *Figure 3*.

Significant differences in LV MAPD were present between dogs with unremodeled hearts from group aAVB and the most severely proarrhythmic remodeled group IVR. Both at baseline and after dofetilide, the lowest values were observed in aAVB (baseline: 234±22, dofetilide: 329±74 ms), while the longest LV MAPD values were observed in IVR (299±44 ms and 439±88 ms). Baseline values in RVA (256±23 ms) were only slightly longer than those in aAVB and comparable to those in HSP (269±16 ms), but after dofetilide this pattern was changed (RVA: 411±70 ms; HSP: 354±28 ms). The baseline LV MAPD values (before start of dofetilide) are plotted in *Figure 6* (x-axis), while T25 values are plotted on the y-axis to provide a direct comparison.

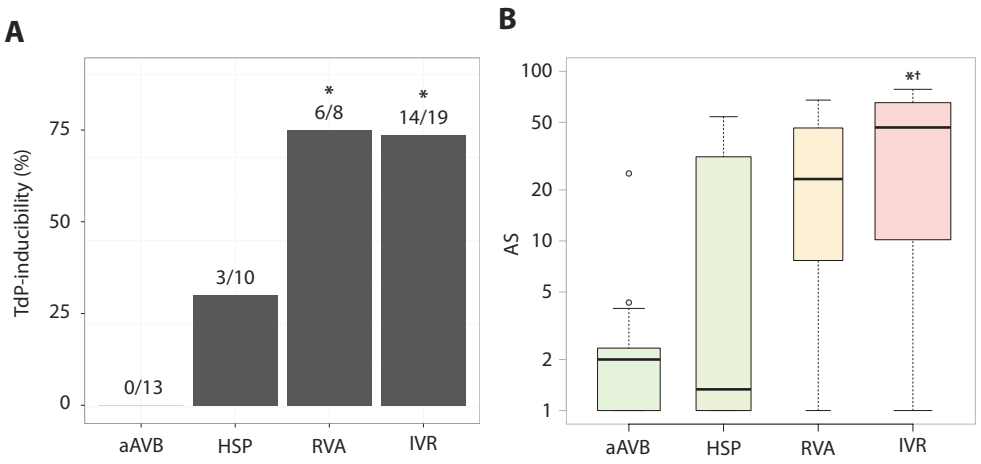


Figure 3

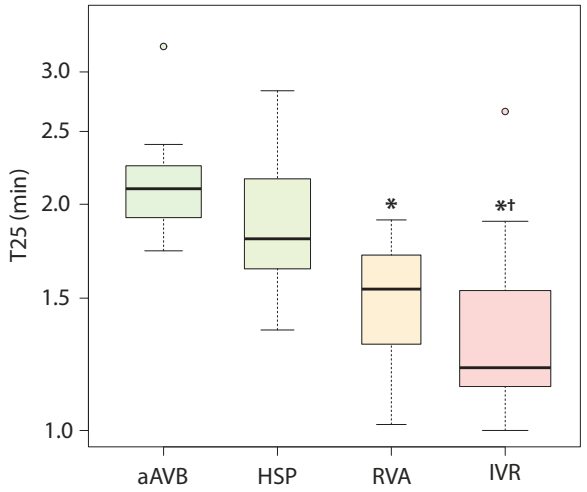
A: Inducibility of repetitive TdP in the different groups (left): dogs with remodeling due to chronic bradycardic, right ventricular apical (RVA) pacing showed a high incidence of TdP, comparable to unpaced chronic AV-block dogs with idioventricular rhythm (IVR). In unremodeled, acute AV-block (aAVB) dogs, inducibility (requiring repetitive TdP) was not observed, while in the high-septal paced (HSP) group only a minority of 30% was inducible.

B: Arrhythmia score (AS) shows the same pattern as inducibility. In the aAVB group the median score was 2, indicating that single ectopic beats were induced in most dogs, but the outliers (circles) indicate that incidentally a TdP episode was observed as well. In most HSP dogs, the arrhythmia score was similar to the AS in aAVB dogs, but a minority of the dogs showed TdP episodes (larger interquartile range of the boxplot). Note: the used colors correlate with the mean AS (green, low; red, high). These colors are also applied in the other figures.

*, P<0.05 vs. aAVB; †, P<0.05 vs. HSP.

Figure 4

The time required to obtain a 25ms-increase of left ventricular monophasic action potential duration after dofetilide (T25) in the different groups. The observed pattern was similar (but inverse) to what has been observed with arrhythmia susceptibility. In both complete AV-block dogs with remodeling due to chronic bradycardic right ventricular apical (RVA) pacing and dogs with chronic idioventricular rhythm (IVR), the 25ms-increase was reached significantly earlier in time as compared to aAVB. High-septal paced (HSP) dogs showed a higher T25 than IVR dogs.



*, P<0.05 vs. aAVB; †, P<0.05 vs. HSP.

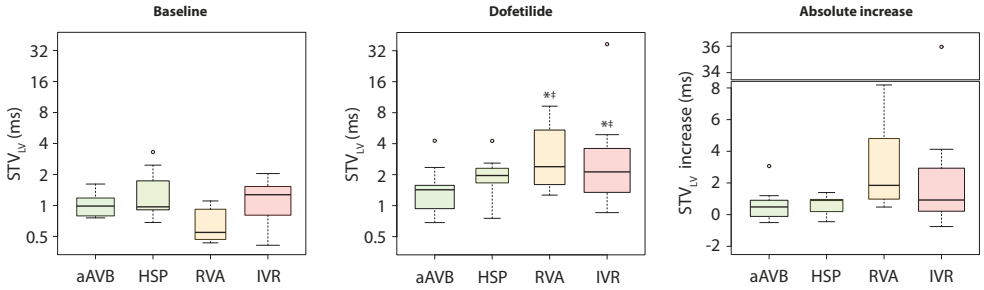


Figure 5

Short-term variability of left ventricular monophasic action potential duration at 80% repolarization (STV), at baseline (left panel) and immediately before the induction of the first ectopic beat after dofetilide (middle panel). The absolute difference (increase) is shown separately at the right side.

At baseline, no significant difference in STV was detected and the pattern of STV values did not resemble the arrhythmia susceptibility (*Figure 2*): the lowest values were found are in the right ventricular apical (RVA) paced dogs, whereas highest values were observed in the high-septal pacing (HS) and idioventricular rhythm (IVR) group. After dofetilide, STV increased significantly in the RVA and IVR dogs, resulting in a pattern that more closely resembled arrhythmogenic outcome.

*, P<0.05 vs. aAVB; †, P<0.05 vs. baseline. Please note the logarithmic scale of the y-axis in panel 1 and 2, which is used because STV shows a lognormal distribution.

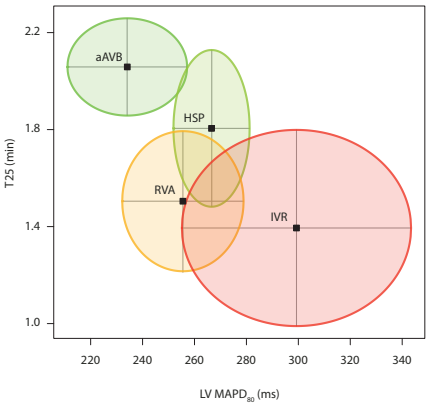


Figure 6

Baseline LV MAPD and the time to obtain a 25ms-increase (T25) after dofetilide, in the four different groups of dogs. Abbreviations are explained in *figure 3*. T25 seems to provide better quantification in the RVA group, in which a relatively short LV MAPD is observed, although arrhythmia susceptibility is high (*figure 3*).

DISCUSSION

Use of arrhythmia score to quantify the severity of repolarization-dependent arrhythmogenesis

In this study, we demonstrated that arrhythmia score captured similar information about arrhythmogenesis as TdP-inducibility if a cut-off value of 7.7 is used. Being a virtually continuous parameter, arrhythmia score may provide a more detailed quantification than the dichotomous parameter inducibility. Especially in serial study designs, use of arrhythmia score may increase

the ability to detect small effect sizes or reduce the required sample size. An example of the former is seen in the group of unremodeled aAVB dogs, in which TdP incidence after dofetilide remained 0%, while arrhythmia score increased significantly to 1.8 [1.0-3.6] (P=0.042). This indicates that dofetilide has a clear, albeit small, proarrhythmic effect in the form of ectopic beats and multiple ectopic beats (and only occasionally, a single TdP). Comparison of arrhythmia scores after dofetilide in HSP and IVR dogs, confirmed the finding of Winckels, et al.[2] that arrhythmia severity was significantly reduced in HSP dogs. In addition, also in our study, which had a slightly expanded sample size but a comparison of more groups, TdP incidence was not significantly different.

Quantification of repolarization reserve

In this study, we demonstrated that the initial increase of LV MAPD after dofetilide, quantified as T25 was a good risk predictor of TdP: the area under the ROC curve was close to 0.8. Although dofetilide infusion was required, the use of a threshold results in early prediction of arrhythmogenic outcome when LV MAPD increase is small.

We also studied alternative parameters to quantify repolarization reserve. For risk prediction, baseline parameters are more important than parameters after administration of dofetilide. However, no difference in baseline STV was found between the differently remodeled groups of dogs and also ROC analysis of the pooled data yielded no predictive value (data not shown). This was unexpected, because it is contradictory to previous studies.[3,11] On the other hand, large differences in baseline STV values have been reported in IVR dogs.[9] This might be related to differences in measurements methods in those studies (e.g. MAPD at 80%, 90% or 100% repolarization, manual or semi-automatic measurements). In the current study, a better standardized and more automatic measurement was used. Variation in baseline STV values may be of less importance if the differences in STV in serial experiments are studied, which is often the case in safety pharmacology studies.[4-6,9] In the current study the increase of STV after dofetilide was highest in the two groups with highest arrhythmic risk (IVR and RVA). In addition, the reached absolute values after dofetilide could be linked to proarrhythmic outcome (*Figure 5*).

The second important baseline parameter, LV MAPD, performed better than STV to predict TdP inducibility, but a limitation of this parameter was already known and confirmed in this study. In the dogs with remodeling due to bradycardic RVA pacing, serial analysis had revealed that the remodeling period was not associated with an increase of LV MAPD [*Chapter 5*]. In the IVR group, the origin of activation is not controlled, thus activation might originate from the right ventricle as well, which may result in a more RVA-like electrophysiological phenotype in some dogs. It has been reported that the focus of idioventricular rhythm mostly originates from within the LV.[2] This may explain the, on average, much longer LV MAPD values combined with the higher standard deviation between dogs and could imply that use of MAPD as predictor of TdP is not optimal in IVR dogs, as long as the focus is not monitored or controlled.

After dofetilide the LV MAPD increased more in the proarrhythmic remodeled dogs, both in

the RVA and IVR group, resulting in a pattern of the LV MAPD values which was more closely linked to the proarrhythmic outcome.

Relevance of remodeling for arrhythmogenesis

It is known that after remodeling due to chronic AV block large inter-individual variation in arrhythmia susceptibility is present, ranging from spontaneous TdP before administration of dofetilide (including a low incidence of sudden cardiac death under conscious conditions) to absence of arrhythmias after dofetilide under general anesthesia.[1,3,11] It remains uncertain whether the variation is caused by differences in remodeling (e.g. amount of bradycardia, and activation pattern) or by pre-existent differences (e.g. genetic factors). In this study we showed that dofetilide increased the arrhythmia score in unremodeled dogs (aAVB), while large differences were already present at aAVB: some dogs showed multiple ectopic beats, or even a single TdP episode, after dofetilide. Eight out of 42 dogs were tested twice, once before (aAVB) and once after remodeling (IVR n= 7, HSP n=1). Correlation analysis of the arrhythmia scores after dofetilide, before and after remodeling revealed a Spearman's correlation of 0.81 (P=0.016), indicating that a correlation is present.

Clinical relevance

Risk estimation of TdP is usually performed using QT interval (corrected for heart rate), but this parameter is cumbersome, because the measurement is difficult,[12] QT prolongation per se is not always proarrhythmic (lack of specificity), but also has insufficient sensitivity.[13-17] It has been shown that QT intervals after challenging repolarization with a drug are higher in patients at risk of arrhythmias, with less overlap in QT intervals than at baseline.[16,18,19]

Our study suggests that also the absolute, initial time-dependent prolongation of repolarization during a standardized infusion of dofetilide (I_{Kr}-block) may be a good predictor of TdP risk and useful to unmask acquired long QT syndrome. This increase might be a better predictor than the QT interval itself, especially if a RVA paced activation is present, but also by preventing substantial QT prolongation after dofetilide administration. However, further study in patients would be required, preferably with additional measures to lower risk of TdP induction, e.g. by using another drug, a lower cut-off value or prophylactic anti-arrhythmic treatment.

Study limitations

During the experiment in groups HSP and RVA the heart rate was controlled by ventricular pacing (VVI mode with a fixed ventricular rate), whereas in the groups aAVB and IVR this was not always performed. Comparison of the parameters (arrhythmia score and electrophysiological parameters) revealed no difference or trend and therefore these groups were merged. Thomsen et al. reported previously that STV was not influenced significantly by the RR variation due to IVR as compared to a fixed paced cycle length and that at paced cycle lengths ≥900 ms STV remained

approximately at a similar level.[5]

The study is a retrospective analysis using experiments from selected groups of animals based on known differences in TdP-inducibility, which hampers the possibility to validate this and therefore this was not an aim of this study.

TdP-inducibility in the CAVB model is determined during a short lasting challenge (5 min infusion of dofetilide, with a 10-15 min observation period). Although it has been reported that repeatability over time is high,[1] this might result in underestimation of proarrhythmic outcome. Programmed electrical stimulation, which has been applied in a number of studies to further study the arrhythmia susceptibility of non-inducible dogs,[6] has never been performed after dofetilide administration.

Dofetilide was infused in five min, but the infusion was always aborted once TdP was induced. This resulted in a lower administered dose in the dogs with inducible TdP, than the dogs without TdP. This may have attenuated the highest arrhythmia scores, but obviously may also have prevented lethal arrhythmias.

Conclusions

Arrhythmia score is a promising method for quantification of arrhythmic response, because it is able to discriminate between inducible and non-inducible animals while providing a more detailed quantification of arrhythmia severity. After dofetilide, the initial increase of LV MAPD provided good risk prediction of dofetilide-induced TdP in groups of differently remodeled AV-block dogs, while the required increase of LV MAPD could be limited to only 25 ms (T25). Advantage over baseline LV MAPD was especially the ability to detect the increased risk of TdP in RVA paced dogs, which showed a short LV MAPD at baseline.

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Chapter 7

Beat-to-beat variability in preload unmasks increased risk of Torsade de Pointes in anesthetized chronic atrioventricular block dogs: a role for mechano-electrical feedback?

Thom R.G. Stams[1,a], Peter Oosterhoff[1,2,a], Atty Heijdel[1], Albert Dunnink[1], Jet D.M. Beekman[1], Roel van der Nagel[1,3], Harold V.M. van Rijen[1], Marcel A.G. van der Heyden[1], Marc A. Vos[1]

[1] *Department of Medical Physiology, Division Heart & Lungs, University Medical Center Utrecht, Utrecht, The Netherlands*

[2] *ICIN-Netherlands Heart Institute, Utrecht, The Netherlands*

[3] *Department of Cardiology, St. Antonius Hospital, Nieuwegein, The Netherlands*

[a] *both authors contributed equally as first author*

Submitted

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ABSTRACT

Rationale: Beat-to-beat variability in ventricular repolarization (BVR) is associated with increased arrhythmic risk in patients and animal models. Proarrhythmic remodeling in the anesthetized dog with chronic AV-block (CAVB) is associated with increased BVR, while I_{Kr}-block with dofetilide further increases BVR and causes TdP arrhythmias.

Objective: We hypothesized that increased BVR in CAVB dogs is caused by an altered response to beat-to-beat variability in preload.

Methods and results: Left ventricular monophasic action potential duration (LV MAPD) was recorded in acute (AAVB) and CAVB dogs, before and after dofetilide infusion. BVR was quantified as short-term variability of LV MAPD. The PQ interval was controlled by pacing: either a constant or an alternating preload pattern was established, which was verified by pressure-volume loop recordings. The effects of the stretch-activated channel blocker streptomycin on BVR and on the arrhythmic response to dofetilide were evaluated in a second CAVB group. Only during alternating preload, BVR was increased after proarrhythmic remodeling (0.45±0.14 AAVB vs. 2.2±1.1 ms CAVB, P<0.01; constant preload: 0.35±0.12 vs. 0.32±0.14 ms, P=NS). At CAVB, dofetilide induced significant proarrhythmia (arrhythmia score: 27[9-62] (median[IQR]) vs. 1[1-2] at AAVB, P<0.05). Preload variability augmented the dofetilide-induced BVR increase at CAVB (+1.5±0.8, vs. +0.9±0.9 ms, P=0.058). In the second group, the increase in baseline BVR by alternating preload (0.3±0.03 to 1.0±0.8 ms, P<0.01) was abolished by streptomycin (0.5±0.2 ms, P<0.05). Furthermore, dofetilide-induced proarrhythmia was attenuated (4[2-13], P=0.07).

Conclusions: In the CAVB dog, the relation between BVR and repolarization reserve originates from altered response to preload variability. Stretch-activated channels appear to be involved both in the mechanisms of BVR and drug-induced arrhythmia.

Keywords: Artificial cardiac pacing; Dofetilide; Mechano-electrical coupling/feedback; Remodeling; Short-term variability of repolarization; Streptomycin; Stretch; Torsade de Pointes; Ventricular arrhythmia

INTRODUCTION

Sudden cardiac death is a major cause of mortality in the general population.[1] Temporal variability of repolarization has been quantified in different ways and shown to be higher in patients with increased risk of arrhythmias.[2-10] The occurrence of repolarization variability is often interpreted as lability of repolarization, resulting from a reduction in the excess capacity of repolarizing currents, known as the 'reduced repolarization reserve' concept.[11] However, the mechanisms underlying repolarization variability are not completely understood. The positive correlation between repolarization variability and arrhythmogenicity is also found in experimental models of cardiac arrhythmia: in animal models sensitive to drug-induced Torsade de Pointes (TdP), beat-to-beat variability of repolarization duration (BVR) is used as a predictor of proarrhythmic side effects in pre-clinical drug screening.[12-14]

In the canine chronic AV-block model (CAVB), an increased TdP-susceptibility is closely linked to ventricular remodeling, which is the result of the chronic bradycardia and altered ventricular activation with AV-dyssynchrony.[15, 16] This remodeling results in repolarization prolongation but also increases BVR, quantified as short-term-variability (STV) of left ventricular (LV) monophasic action potential duration (MAPD) [STV_{MAPD}].[15] Drugs that further delayed repolarization and induced TdP yielded a strong increase in BVR prior to arrhythmias occurrence, whereas non-arrhythmogenic drugs, despite QT prolongation, did not increase BVR.[17-20] Besides myocardial remodeling effects, extra-cardiac factors like preload may also contribute to BVR. Preload induced stretch, and stretch in general are known to influence cardiac electrophysiology.[21, 22] This may be especially true in the AV-block dog in which AV-dyssynchrony leads to uncontrolled beat-to-beat changes in ventricular preload. We hypothesized that this variability in preload, is required to provoke repolarization variability in a situation of reduced repolarization reserve.

In the current study, we investigated the influence of preload variability on BVR at different levels of repolarization reserve (remodeling and I_{Kr} block). Furthermore, we evaluated the effect of stretch activated channel (SAC) block on both BVR and the induction of TdP by I_{Kr}-block. Results show that preload variability is required for changes in BVR after pro-arrhythmic remodeling or I_{Kr}-block, and reduction of stretch activated current (I_{SAC}) will attenuate both baseline BVR and arrhythmic response to dofetilide.

METHODS

Animal handling

All experiments were approved by the committee for experiments on animals of Utrecht University and animal handling was in accordance with the Dutch Law on Animal Experimentation and the European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European Union Directive 86/609/EEC).

All experiments were performed under general anesthesia. Premedication consisted of an intra-

muscular injection with acepromazine, methadone and atropine (10 mg, 10 mg and 0.5 mg respectively). Anesthesia was induced with sodium pentobarbital (25 mg/kg i.v.) and maintained with isoflurane (1.5% in O_2 and N_2O, 1:2). Tidal volume of mechanical ventilation was adjusted to maintain an expired CO_2 concentration between 3.5 and 4.5%.

Preparation

To create third degree AV-block, radiofrequency energy was applied to the proximal bundle of His.[23] In animals selected for repeated dofetilide testing at CAVB, a custom pacing electrode was implanted transmurally in the LV apex using a right thoracotomy, through the fourth or fifth intercostal space. This electrode was electrically isolated except for the most distal part, allowing sub-endocardial stimulation from the same location during repeated experiments. During PV-loop measurement the LV MAP catheter was used for ventricular stimulation. Atrial stimulation was performed using a screw-in pacing electrode (5076, Medtronic Inc. Minneapolis, USA) or a temporary pacing lead placed in the right atrium.

Data Acquisition

At the start of experiments a MAP catheter (Hugo Sachs Elektronik, Germany) was placed onto the left ventricular endocardium and signals were digitized and stored on a PC, simultaneous with a regular 6 lead ECG. Pressure-volume (PV) loop recordings were acquired using a combined PV conductance catheter (CD Leycom Inc., Zoetermeer, Netherlands), connected to a monitor (Sigma M, CD Leycom) and a PC with dedicated software (ConductNT, CD Leycom).

Pacing induced preload variability

Asynchrony of atrial and ventricular contractions are typical for the AV block model and results in beat-to-beat changes in preload. We designed a pacing protocol to control atrial and ventricular activation separately, allowing us to turn preload variability on or off. An external pacemaker (PK5, Vitatron, Arnhem, The Netherlands) was adapted to perform stimulation of atria and ventricles at different pacing rates, starting at a pre-set AV delay (=PQ interval on ECG) of 150 ms (similar to sinus rhythm). This allowed control of PQ interval, and thereby preload, while maintaining the ventricular bradycardia required for TdP induction at 1000 ms.[24] The pacing sequences are illustrated in *Figure 1A*: a constant PQ interval (constant preload) was obtained when the RR:PP-ratio was 2.0 (or 3.0), while an RR:PP-ratio of 2.5 (or 3.5) provoked an alternating PQ interval (maximal beat-to-beat variation), resulting in maximum pacing induced preload variability (*Figure 1B*).

Because ventilation may cause alterations in preload as well, measurements were preferably performed with ventilation temporarily switched off. Only the effect of dofetilide, where STV_{MAPD} is evaluated just before first ectopic activity, was evaluated with ventilation on. Therefore, baseline recordings of BVR at constant and alternating PQ were performed both during mechanical

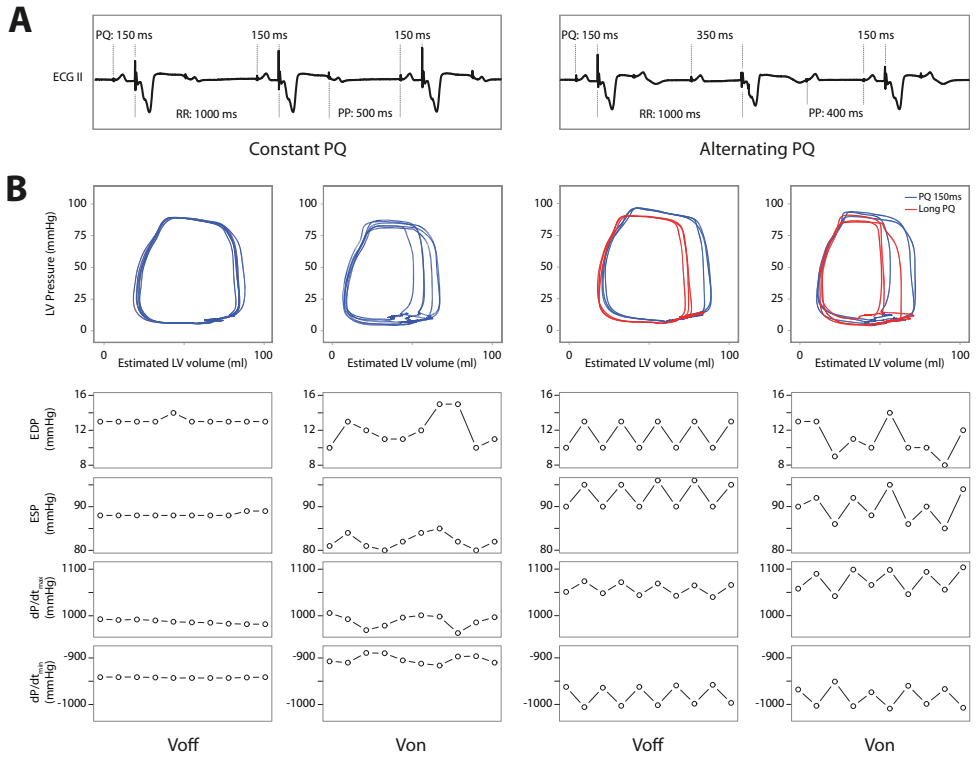


Figure 1

A: Example ECG representation of the used pacing protocols. Stimulation of atria and ventricles at different heart rates allows control of atrio-ventricular timing, while preserving the ventricular bradycardia required for TdP induction. When the paced VV (RR) interval is an exact multiple of the AA (PP) interval, each ventricular activation is preceded by the same PQ interval (left tracing), whereas a RR:PP-ratio of 2.5 results in alternation of the PQ interval (right tracing). Printed numbers are resulting PP, PQ and RR interval.

B: Pressure volume (PV) loop recordings, each showing six consecutive beats during different protocols in which preload variability was acutely controlled. The lower four panels show parameters derived from the pressure recording, which was calibrated, over ten consecutive beats. Clearly visible is the minimal mechanical variation during constant PQ and ventilation off (Voff; most left PV loop). Ventilation alone (PV loop 2) results in cyclical variation of EDP but also the other mechanical parameters and volume. The cycle length corresponds to the ventilation frequency of 12/min. During alternating PQ the alternating pattern dominates, also if ventilation is switched on (PV loop 4). Note that the long PQ interval result in an only temporary increase of volume and pressure (most clearly visible during Voff; PV loop 3; red curves lower right part), which can be explained by backflow of blood before ventricular contraction starts.

ventilation (Von), and while mechanical ventilation was ceased for 30 s (Voff), resulting in four modes of preload variability (*Figure 1B*). Volume controlled mechanical ventilation was set at a frequency of one fifth of the heart rate.

Challenges on repolarization reserve: cardiac remodeling and I_{Kr} block

To compare the effect of preload variability on BVR after various challenges on repolarization reserve eleven animals were used (age 1.3±0.3 years, body weight 22±3 kg, six males, Marshall, NY, USA). Baseline BVR was measured at acute AV-block in six dogs. Five animals also received a dofetilide challenge (0.025 mg/kg in 5min) to test susceptibility to TdP after I_{Kr} block. Measurements with dofetilide at AAVB were randomized to with or without pacing induced preload variability.

Serial experiments in the remodeled heart were performed in seven animals at 3 and 5 weeks CAVB. Both the effects of preload variability on BVR at baseline and after administration of dofetilide were tested; once with pacing induced preload variability (alternating PQ) and once without (constant PQ), in a random order.

After the start of dofetilide infusion the ECG was monitored for TdP, which was defined as a polymorphic ventricular tachyarrhythmia of at least five beats characterized by a twisting shape of the QRS complexes around the isoelectric line. Dofetilide infusion was stopped after the first detected TdP episode. If ventricular tachycardia lasted more than 10 s, electrical cardioversion was applied (10-150-200 J biphasic).

Quantification of preload variability and effect of stretch activated channel block

PV-loops were recorded in order to quantify the pacing induced preload variability, with and without mechanical ventilation, simultaneous with LV MAP, in an additional set of nine CAVB dogs (median 9 [7-14] weeks after AV-block). In the same experiment the effect of SAC block by streptomycin on baseline BVR and arrhythmic response to dofetilide was tested; during pacing with alternating PQ, streptomycin was infused (40 mg/kg in 5 min i.v.), followed 10 min later by dofetilide (0.025 mg/kg in 5 min).

Data analysis

MAP duration was measured at 80% repolarization (MAPD) using custom software (Matlab, Mathworks, Natick , USA), provided that signal quality was sufficient (plateau amplitude of at least 10 mV and a stable isoelectric line and morphology). BVR was quantified as STV of MAPD over 30 beats: $STV=\sum_{1..30}|D_n-D_{n-1}|/(30\cdot\sqrt{2})$, where D represents LV MAPD. QT intervals of ECG (lead II) were determined manually using onscreen calipers (ECG-AUTO, EMKA Technologies, Paris, France).

Arrhythmic outcome was quantified by combining the number of ectopic beats, episodes of TdP and defibrillations into a single arrhythmia score. This score was introduced recently and provides more detail than the dichotomous outcome inducibility of TdP [*Chapter 6*].

For PV-loop recordings a pseudo-calibration was applied to the volume signal by assuming the following rough estimates: EDV=65mL, ESV=15mL, EF=77%, based on previously published results performed by our group.[25] The markers that indicate the end-diastolic and end-systolic

pressure/volume were all checked and manually adjusted if necessary. Means and STV were calculated using 30 consecutive beats.

Statistical analysis

Data are expressed as mean ± standard deviation (SD), or median and interquartile range (IQR, within square brackets), unless noted otherwise. Pairwise comparisons were performed using *t*-tests for normal distributed variables and Wilcoxon rank-sum tests for non-normally distributed variables, including arrhythmia score. For more than two groups, one or two-way repeated measures ANOVA was used (after logarithmic transformation, if necessary), with post-hoc Bonferroni *t*-tests, when appropriate. If the data in one-way ANOVA did not meet requirements of normal distribution Friedman test was used with post hoc Wilcoxon signed rank tests in pairwise comparisons. Two-tailed tests were used and a P-value of less than 0.05 was considered significant.

RESULTS

Variation in PQ results in preload variability

Control over preload variability through pacing was quantified with PV loop recordings (individual example in *Figure 1B*) using STV calculations of LV mechanical parameters (lower part *Table 1*): While ventilation was off, alternating PQ resulted in significant increase in STV of all pressure-derived parameters compared to constant PQ pacing, showing that we have ample control of preload variability through our stimulation protocols. When ventilation was on the differences remained significant for six out of seven parameters. Looking at individual beats during alternating PQ (ventilation off), all hemodynamic parameters were consistently and significantly decreased during the beats with long PQ (>350 ms) compared to beats with short PQ (150 ms). Apart from a small decrease in EDP at alternating PQ, the 30-beats averages of the mechanical parameters were not influenced by the four modes of preload variability (upper part *Table 1*).

Increased BVR after proarrhythmic remodeling requires preload variability

The ventricular remodeling due to CAVB at idioventricular rhythm was associated with QTc prolongation: 327±19 ms in AAVB and 440±46 ms in CAVB dogs (P=0.001). At AAVB, LV MAPD and STV_{MAPD} were not affected by changes in preload variability (*Figure 2A and 2B*, open squares). Electrical remodeling increased baseline STV_{MAPD} significantly, but only during pacing with alternating PQ interval (*Figure 2B*, black squares). We consistently observed a longer LV MAPD in beats with short PQ (highest preload). Mechanical ventilation had no significant effect on STV_{MAPD} either before or after remodeling (*Figure 2B*).

To confirm that the effect on STV_{MAPD} at CAVB was caused by PQ variability and not by the PP interval itself, we recorded STV_{MAPD} at a range of PP intervals, resulting in PP:RR ratios from

Table 1. Parameters derived from pressure-volume loop recordings during the different protocols that were used to control preload variability.

	Constant PQ		Alternating PQ	
	Ventilation off	Ventilation on	Ventilation off	Ventilation on
EDP, mmHg	14.1 ± 4.1	14.4 ± 4.1	13.3 ± 4.0*	13.2 ± 3.9 †
ESP, mmHg	92 ± 6	91 ± 6	90 ± 5	89 ± 4
dP/dt_{max}, mmHg/s	966 ± 158	960 ± 161	965 ± 150	969 ± 158
dP/dt_{min}, mmHg/s	-893 ± 147	-885 ± 144	-896 ± 127	-891 ± 119
EDV, ml	80 ± 23	75 ± 30	82 ± 28	77 ± 31
ESV, ml	34 ± 22	29 ± 26	34 ± 25	30 ± 28
SW, ml·mmHg	4047 ± 712	3958 ± 787	4076 ± 688	3991 ± 761
STV_{EDP}, mmHg	0.1 ± 0.0	0.9 ± 0.2 ***	1.2 ± 0.7 ***	1.3 ± 0.3
STV_{ESP}, mmHg	0.3 ± 0.3	1.1 ± 0.4 ***	3.0 ± 1.1 ***	3.0 ± 1.0 †††
$STV_{dP/dt\ max}$, mmHg/s	1.6 ± 0.6	5.0 ± 3.7 ***	16.8 ± 8.3 ***	17.0 ± 9.3 ††
$STV_{dP/dt\ min}$, mmHg/s	1.2 ± 0.5	4.2 ± 1.6 ***	13.1 ± 7.5 ***	15.1 ± 8.4 †
STV_{EDV}, mmHg	0.8 ± 0.3	2.2 ± 1.4 ***	3.7 ± 2.2 ***	3.6 ± 1.7 ††
STV_{ESV}, mmHg	0.7 ± 0.3	1.2 ± 0.5 ***	2.7 ± 1.2 **	2.5 ± 1.2 †
STV_{SW}, mmHg	83 ± 23	157 ± 124 ***	370 ± 221 ***	336 ± 215 ††

The four different protocols are explained by an individual example in *Figure 1*. Values in italics are estimates only, because a pseudo-calibration was used for the volume signal. During the measurements the ventricle was paced at a fixed rate of 60/min, while the PQ was either constant (150 ms) or alternating (between 150 and >350 ms; see also *Figure 1*). Measurement were performed with and without mechanical ventilation; EDP, end-diastolic pressure; ESP, end-systolic pressure; dP/dt_{max}, maximum rate of rise of LV pressure; dP/dt_{min}, minimum rate of fall of left ventricular pressure; EDV, end-diastolic volume; ESV, end-systolic volume; SW, stroke work. */**/*** P<0.05/<0.01/<0.001 vs. Ventilation off during constant PQ. †/††/†††, P<0.05/<0.01/<0.001 vs. Ventilation off during constant PQ. No differences were found between Ventilation on and Ventilation off during alternating PQ.

2.0 to 3.5 (*Figure 2C*). The local minima at constant PQ (ratios 2.0 and 3.0) and local maxima at alternating PQ (ratios 2.5 and 3.5), but no trend in STV from left to right (decreasing PP), show that STV is indeed the result of the variation of PQ interval and not of PP interval per se.

Preload variability augments STV increase after dofetilide

Consistent with previous publications, administration of dofetilide induced an arrhythmic response in CAVB, but not in AAVB: pooled results for experiments with constant and alternating PQ (*Figure 3A*) show the arrhythmia score was significantly higher in CAVB dogs (AAVB: 1 [1-2], (n=5); CAVB: 27 [9-62] (n=7); P=0.005). When comparing the serial experiments (constant and alternating PQ) at CAVB, it is clear that the arrhythmia score was not changed (16 [7-37] at constant PQ; 25 [2-48] at alternating PQ, n=6; P=0.94; *Figure 3B*).

Dofetilide increased LV MAPD equally during constant PQ and alternating PQ, and no sig-

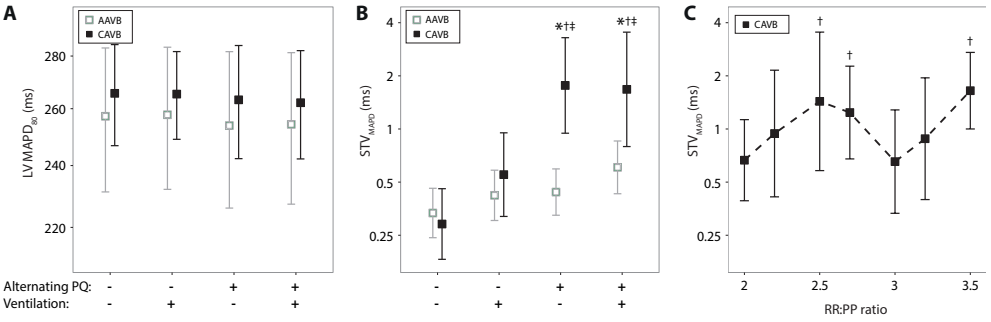


Figure 2

The effect of beat-to-beat changes in preload caused by PQ variation and mechanical ventilation on (A) left ventricular monophasic action potential duration (LV MAPD) and (B) short-term variability of LV MAPD (STV_{MAPD}) at acute and chronic AV-block (AAVB, CAVB).

*, P<0.05 vs. AAVB; †, P<0.05 vs. constant PQ and ventilation off; ‡, P<0.05 vs. constant PQ and ventilation on.

C: Effect of variations in the ratio of ventricular to atrial cycle length (RR:PP ratio). The atrial cycle length was stepwise shortened, while maintaining the fixed RR interval of 1000 ms. Maxima for STV_{MAPD} are seen at alternating PQ (ratio 2.5 and 3.5), whereas minima are observed at 2.0 and 3.0, where PQ is constant.

†, P<0.05 vs. 2.0.

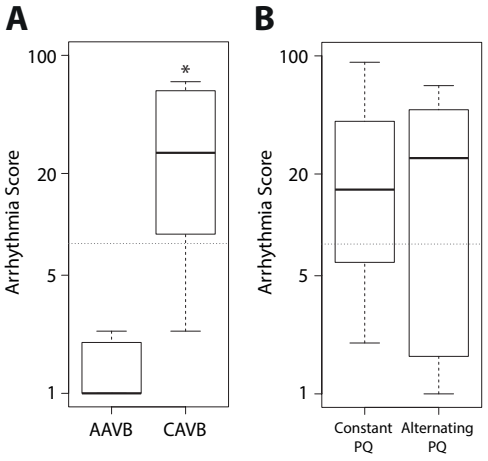


Figure 3. Effect of dofetilide on arrhythmia score (AS).

A: In unremodeled dogs at acute AV-block (AAVB; n=5), dofetilide was administered during either alternating (n=3) or constant PQ (n=2). Torsade de Pointes was not observed, resulting in a low AS. At chronic AV-block (CAVB; n=7) dogs were tested twice, at both alternating PQ and a constant PQ (1 missing). The mean AS of these 2 experiments was significantly higher than at AAVB.

B: Serial analysis of the arrhythmia score after dofetilide in experiments during alternating and constant PQ revealed no (trend towards) a difference in AS. Note: the red dotted lines mark the best cut-off value to discriminate TdP inducible vs. non-inducible animals, based on the results of a previous study [*Chapter 6*].

nificant differences were present between the groups (*Figure 4A*). However, alternating PQ augmented the increase in STV_{MAPD} by dofetilide (+1.5±0.8 and +0.9±0.9 ms; P=0.058, *Figure 4B*). In *Figure 4C* an individual example is shown; clearly visible are the oscillations in MAPD corresponding to alternating PQ and mechanical ventilation, that are augmented after dofetilide.

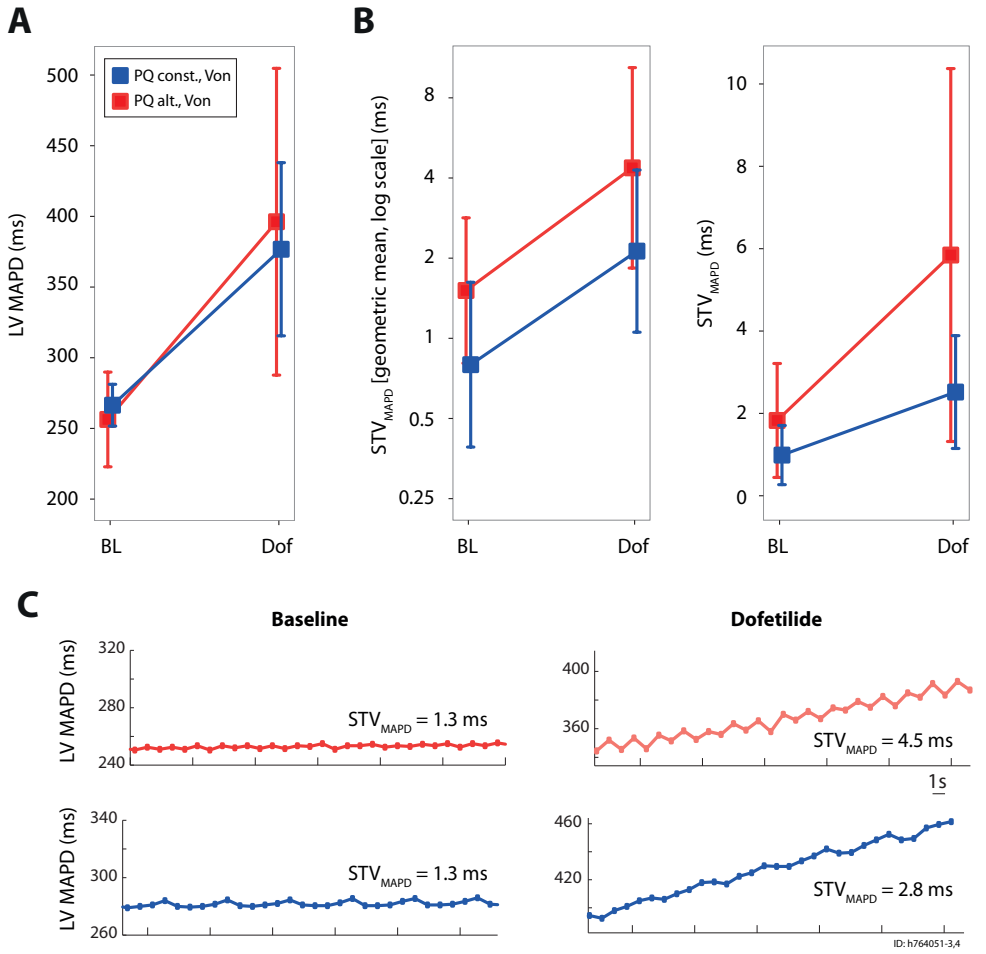


Figure 4

This figure shows the electrophysiological effects of dofetilide during constant PQ (blue) and alternating PQ (red) (Exp. 2 and 3).

A: Immediately after complete infusion of dofetilide (or before induction of the first ectopic beat), LV MAPD is increased equally during constant and alternating PQ.

B: The effect of dofetilide on STV_{MAPD} shown on as geometric mean */ geometric SD, on a logarithmic scale (left panel) or mean±SD (right panel). Due to the lognormal distribution of STV_{MAPD}, mean±SD may not provide the optimal visualization (but this is the most common representation in the literature). The increase of STV_{MAPD} was larger during alternating PQ than during constant PQ (P=0.058).

C: Individual example showing traces of LV MAPD used for STV_{MAPD} calculation at baseline and after dofetilide (each 31 consecutive beats). Clearly visible is that dofetilide administration increased the amplitude of the alternating MAPD after dofetilide (red lines) in this dog.

Streptomycin abolishes response of BVR to preload variation and attenuates arrhythmic response to dofetilide

The response of STV_{MAPD} to alternating PQ at baseline was abolished by streptomycin (individual example in *Figure 5*): from 0.3±0.03 at constant PQ to 1.0±0.8 ms at alternating PQ (P<0.01) back to 0.5±0.2 ms at alternating PQ after streptomycin (P<0.05 vs. baseline alternating, NS vs. baseline constant). Streptomycin had no effect on the variability of the LV mechanical parameters, while the 30 beat averages only decreased slightly after the administration (*Table 2*).

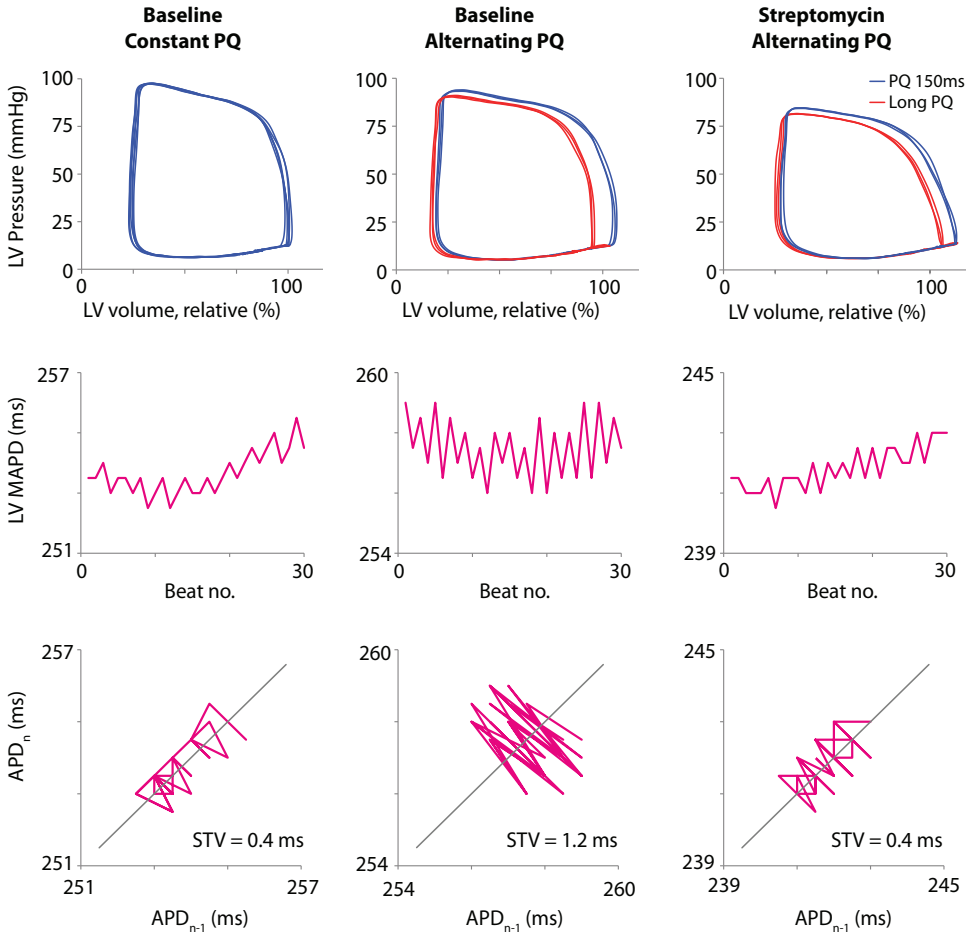


Figure 5. Individual example illustrating the effect of streptomycin on the pressure-volume loop and on STV-MAPD in a dog with chronic AV-block.

After streptomycin (right) beat-to-beat alterations in preload are maintained whereas STV_{MAPD} is reduced to the same level as seen during alternating PQ before streptomycin administration (left). All measurements performed with mechanical ventilation off.

Pretreatment with streptomycin showed a strong trend towards reduction of the arrhythmia score following dofetilide infusion: 27 [9-62] vs. 4[2-13] (P=0.07, *Figure 6*). However, this could not prevent an increase of STV_{MAPD}, from 0.6±0.2 to 4.0±3.3 ms (P=0.006), and is similar to the value obtained in the group that received only dofetilide.

Table 2. Mechanical and electrophysiological effects of preload variability and the effect of streptomycin.

	Baseline Constant PQ	Baseline Alternating PQ	Streptomycin Alternating PQ
LV MAPD, ms	256 ± 19	255 ± 25	245 ± 22
STVMAPD, ms	0.3 ± 0.1	1.2 ± 0.7 *	0.5 ± 0.2 †
EDP, mmHg	14.1 ± 4.1	12.5 ± 3.6	12.4 ± 3.7
ESP, mmHg	92 ± 6	87 ± 7	79 ± 9 *
dP/dt_{max}, mmHg/s	966 ± 158	913 ± 166	780 ± 137 *†
dP/dt_{min}, mmHg/s	-893 ± 147	-864 ± 135	-743 ± 123 *†
EDV, ml	80 ± 23	67 ± 3	71 ± 4
ESV, ml	34 ± 22	22 ± 6	27 ± 8
SW, ml·mmHg	4047 ± 712	3815 ± 734	2990 ± 517
STV_{EDP}, mmHg	0.1 ± 0.0	1.2 ± 0.6 *	0.9 ± 0.5 *
STV_{ESP}, mmHg	0.3 ± 0.3	2.8 ± 0.9 *	2.8 ± 0.9 *
$STV_{dP/dt\ max}$, mmHg/s	1.6 ± 0.6	16.5 ± 8.4 *	22.0 ± 17.5
$STV_{dP/dt\ min}$, mmHg/s	1.2 ± 0.5	13.6 ± 7.1 *	12.1 ± 11.2
STV_{EDV}, mmHg	0.8 ± 0.3	3.8 ± 2.2 *	3.3 ± 1.6 *
STV_{ESV}, mmHg	0.7 ± 0.3	2.8 ± 1.2 *	1.9 ± 1.1 *
STV_{SW}, ml·mmHg	83 ± 23	350 ± 238 *	313 ± 163 *

Mechanical parameters (pressure and volume) were derived from left ventricular pressure volume loop recordings. LV MAPD, left ventricular monophasic action potential duration at 80% repolarization; STV_X, short term variability of parameter *X*. Other abbreviations are explained in *Table 1*. *, P<0.05 vs. baseline during constant PQ; †, P<0.05 vs. baseline during alternating PQ.

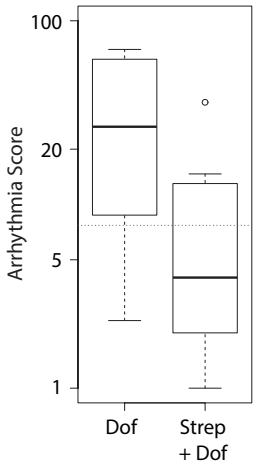


Figure 6

Preventive effect of streptomycin against dofetilide-induced arrhythmia, quantified as arrhythmia score, in dogs with chronic AV-block (CAVB; n=9), compared with CAVB dogs that were administered dofetilide alone (*Figure 3A*). Streptomycin was (partially) anti-arrhythmic (P=0.07 vs. control).

Dof, dofetilide; Strep, streptomycin

DISCUSSION

The most important findings of this study are: (1) in the chronic AV-block dog model, preload variability due to variability of PQ interval is an important contributor to BVR; (2) the variation in preload is a prerequisite to obtain a significant increase of STV_{MAPD} after proarrhythmic remodeling and it augments the STV_{MAPD} increase after dofetilide; (3) the effect of preload variability on baseline STV_{MAPD} is abolished by streptomycin infusion, while mechanical variation is maintained; (4) arrhythmia score after dofetilide application is independent of pacing induced preload variability, but is reduced by streptomycin.

These results confirm the association between STV_{MAPD} and proarrhythmia and suggest involvement of SAC in arrhythmogenesis of TdP in the CAVB dog.

BVR in patients and experimental models

The CAVB dog is characterized by: (a) chronic altered ventricular activation during the remodeling period, due to idioventricular rhythm with unpredictable origin(s); (b) AV-dyssynchrony resulting in beat-to-beat variation of PQ interval; and (c) bradycardia. After 'preconditioning' with premedication/anesthetics, dofetilide causes repetitive TdP episodes in about 75% of the CAVB dogs associated with severe QT prolongation.[26, 27]

Previous publications have revealed the relation between ventricular remodeling, STV_{MAPD} in baseline and the susceptibility to drug-induced arrhythmias in the anesthetized CAVB dog: Thomsen et al. showed that in these CAVB dogs, baseline STV_{MAPD} was increased in comparison to unremodeled hearts from dogs in sinus rhythm or in AAVB. The highest values were measured in animals in which TdP arrhythmias were inducible.[15] However, in these studies using the non-paced AV-block dog the preload variability has not been controlled and as a consequence may vary over time and between individuals. This may have caused uncontrolled peaks and troughs in preload variability and thereby in STV_{MAPD}.

Clinically, Hinterseer et al. confirmed the relation between baseline STV of repolarization and arrhythmia susceptibility in patients with (1) a history of drug-induced TdP; (2) inherited long QT syndrome; and (3) heart failure.[6, 7, 28] A relation between BVR and ventricular arrhythmias has been demonstrated in more clinical studies, as described in the introduction.

Furthermore, our group and others showed in animal models that drugs that induce TdP will lead to increased STV values, irrespective of prolongation of QT or LV MAPD.[12, 13, 27, 29, 30] Interventions that suppress or prevent TdP, also lead to a decrease in STV_{MAPD}.[11, 20, 24]

Mechanistic link between BVR and preload variability

In this study, we confirmed that baseline STV_{MAPD} is increased after remodeling, but only if preload variability is present, and this effect was abolished after I_{SAC} block. Thus, to unmask the increased risk of TdP with baseline STV_{MAPD}, a (mechanical) 'challenge', here in the form of

preload variability is essential. The simplest explanation would be that similar I_{SAC} would have a larger effect on repolarization when repolarization reserve is reduced through electrical remodeling or dofetilide. One might then however expect that TdP would be inducible in the unremodeled heart also using sufficient I_{Kr} block, which was not the case. An alternative explanation can be that I_{SAC} is increased in cardiac hypertrophy, as found by Kamkin et al.,[31] which would be in line with the higher sensitivity of STV_{MAPD} to preload variability in the remodeled heart, both at baseline and after dofetilide.

As a third factor the relative timing of electrical and mechanical systole may play a role in BVR: in the CAVB dog, dofetilide prolongs repolarization, but does not delay the interval from QRS onset to end of relaxation, reversing the physiological situation where mechanical relaxation occurs after repolarization (a negative 'electromechanical window' [see also *Chapter 4*]).[32] Isovolumic relaxation and filling during early diastole, which are associated with increased local differences in stretch, now overlap with the vulnerable phase of repolarization; allowing resulting I_{SAC} to locally affect the repolarization process. Again, this would require an additional factor like increased I_{SAC} after remodeling, because the relative timing of electrical and mechanical systole after dofetilide is not different in experiments in CAVB with versus without induction of TdP, despite the difference in arrhythmic response.[33]

Specificity of SAC blockade by streptomycin

To elucidate a role of SAC in BVR, we tested the effect of I_{SAC} block by streptomycin (40mg/kg/5min i.v., target plasma level 200 μM) in CAVB dogs. While maintaining the mechanical beat-to-beat variation during alternating PQ, STV_{MAPD} returned to a level that had been observed at baseline during constant preload.

Streptomycin is a non-specific blocker of SAC (IC_{50}: 200 μM). At higher concentrations the drug may also block other channels, most importantly the L-type calcium channel (IC_{50} of I_{CaL}: 2 mM).[34-36] Block of I_{CaL} is relevant because this has shown to be anti-arrhythmic in the CAVB model, but only at a dose where a negative inotropic pressure is observed.[37] To explore the possibility of I_{CaL} block in our experiments, we evaluated whether a negative inotropic effect was present: we found that ESP was reduced by 9%. This was associated with a 4% reduction of LV MAPD. Bourgonje et al.[37] have observed an anti-arrhythmic effect of verapamil at a dose that seemed to results in a larger reduction of ESP, but this drug also blocks other channels besides I_{CaL}.

However, negative inotropic response may be a poor marker for block of non-stretch activated current. Stretch activated current is not limited to effects via specialized stretch-activated ion channels. For example also I_{CaL} and I_{Na} have been reported to be directly mechanosensitive.[38, 39] Thus, even perfect block of stretch-sensitive currents might result in negative inotropic effects. In this study we cannot completely separate the effect of I_{SAC} block versus (non-stretch sensitive) I_{CaL} block. Despite these limitations, we think the effect of streptomycin on baseline BVR and arrhythmic response, combined with the association between baseline BVR and arrhythmogenic-

ity, is a first indication that I_{SAC} may be involved in induction of drug induced arrhythmia.

Relevance of mechanical variation for STV and arrhythmogenesis of TdP

Based on the predictive value of baseline STV_{MAPD} for TdP, one might expect an anti-arrhythmic effect of low preload variability, because it reduces STV_{MAPD}. However, a difference in arrhythmia severity after dofetilide, between the two serial experiments with constant PQ or alternating PQ (Ventilation on), was not observed: under both conditions ectopic beats and TdP episodes were induced in most dogs (*Figure 3B*). However, this does not tell us if the preload variation plays a role in the progression from ectopic activity to TdP, as we no longer control preload variability after ventricular ectopy.

To explore whether mechano-electrical feedback is involved, dogs were pre-treated with the SAC blocker streptomycin, before dofetilide was administered. This partially prevented the dofetilide-induced TdP, more directly than suppression of baseline STV_{MAPD}, suggestive of involvement of mechano-electrical feedback in arrhythmogenesis. However, the precise role of SAC in TdP induction remains to be elucidated.

The proarrhythmic effect of drugs in relation to BVR has also been studied in isolated cardiomyocytes. Here the arrhythmic endpoint is often occurrence of early or delayed afterdepolarizations (EADs, DADs), and BVR is determined using transmembrane action potentials, measured using microelectrodes. Evidence exists that in vivo, electrotonic coupling of cells through connexins can suppress both BVR and EAD formation.[40] EAD formation in vivo may be a stochastic process, requiring local synchronization of EADs before an ectopic beat is induced (source-sink mismatch).[41] Well-timed stretch during late repolarization might provide this synchronizing inward current that triggers ectopic beats in the intact heart. Under physiological conditions, stretch can modify the action potential differently depending on the characteristics of the stretch and the timing: it has been shown that a static and non-excessive volume increase results in shortening of the action potential near the plateau level, whereas the end of the action potential can prolong, with an 'afterdepolarization'-like prolongation.[21] Short-lasting stretch can have different effects, depending on the timing: stretch depolarizes the membrane when applied during diastole or late during the action potential. The depolarization can result in an afterdepolarization-like event, but can also result in triggering of an ectopic beat, depending on both the amplitude of the stretch and the velocity of the stretch application. In contrast, if stretch is applied during the plateau phase repolarization is enhanced during application.[42] In combination with the negative electromechanical window mentioned earlier (repolarization delayed beyond mechanical relaxation), this might promote formation of early afterdepolarizations and induction of TdP.[21, 43] Kim et al. showed that in rats either a sudden increase or a reduction of stress could trigger ectopic beats. The sensitivity to stretch induced ectopy was increased after hypertrophy and could be suppressed with streptomycin.[44]

Clinical applications of BVR for evaluation of proarrhythmic risk

Our data confirm the usefulness of STV_{MAPD} for arrhythmic risk prediction, but with the important finding that a controlled challenge, which can be induced by preload variability, is required. This opens perspective to apply changes in preload to increase the predictive value of STV of repolarization for arrhythmias, in humans and animal models. Hansen et al. recently demonstrated that in healthy humans without bradycardia, activation recovery intervals at different sites in the heart varied with respiration, with an amplitude that often exceeded 5 ms.[45] ECG recording during controlled respiration using a quantification method more sensitive to the slow variation corresponding to respiration may be one of the least invasive ways. In patients with an implanted pacemaker or during an electrophysiological study, a dedicated pacing protocol provoking changes in preload, while monitoring electrogram recordings may be useful for arrhythmic risk prediction.

Study limitations

Although in our study the pacing induced variation in PQ was similar in AAVB and CAVB we cannot rule out that hemodynamic or structural changes at CAVB play a role in the altered response of repolarization. For instance, the diastolic inner diameter of the left ventricle is increased in CAVB, changing the relation between ventricular pressure and the stretch the individual myocytes are subjected to.[46] Therefore, the definition of an equivalent variation in preload is not straightforward and factors other than repolarization reserve may play a role in the increased response of LV MAPD to PQ variation after remodeling.

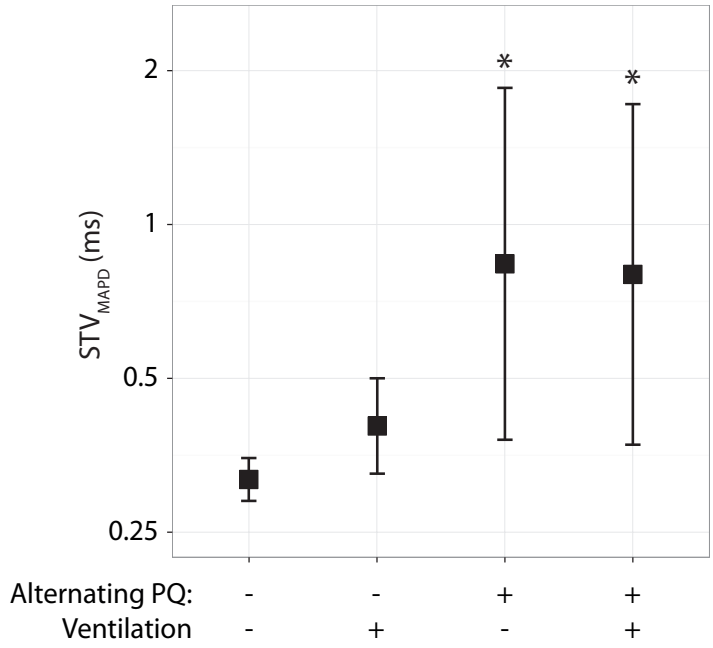
Conclusions

In the anesthetized AV-block dog with a fixed paced heart rhythm, underlying mechanism of BVR is an augmented response of repolarization to beat-to-beat changes in cardiac preload when repolarization reserve is reduced. The increase in BVR after proarrhythmic remodeling and just before TdP-induction with dofetilide depends on preload variability. Mechano-electrical feedback through SAC appears to be involved because streptomycin abolished the effect of preload variability on STV_{MAPD} at baseline. The anti-arrhythmic effect of streptomycin pretreatment against dofetilide-induced TdP may indicate a role of mechano-electrical feedback in arrhythmogenesis of TdP as well. For use of BVR as an arrhythmic marker, applying controlled changes in preload may be a safe way to improve predictive value, also in a clinical setting.

Acknowledgement

We thank Dr. Jan Schreuder, Dr. Tycho van der Spoel and Drs. Geert van Hout for technical assistance with PV-loop recordings.

SUPPLEMENT



Supplemental Figure S1

Effect of alternating PQ and mechanical ventilation on STV of LV MAPD in the dogs used for PV-loop measurement. Data are presented as geometric mean */ geometric SD. The y-axis has a logarithmic scale. In post hoc analysis, all data were compared vs. alternating PQ during mechanical ventilation off; *, P<0.05.

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Chapter 8

Chronic dyssynchronous ventricular activation is proarrhythmic and this is reversible with cardiac resynchronization therapy

Thom R.G. Stams[1], Mathias Meine[2], Jet D.M. Beekman[1], Roel van der Nagel[1], Marc A. Vos[1]

[1] *Department of Medical Physiology, Division Heart & Lungs, University Medical Center Utrecht, Utrecht, The Netherlands*

[2] *Department of Cardiology, Division Heart & Lungs, University Medical Center Utrecht, Utrecht, Netherlands*

In preparation

8

ABSTRACT

Introduction: Dyssynchronous left ventricular activation (DVA) reduces pump function and is associated with ventricular remodeling, whereas cardiac resynchronization therapy (CRT) is applied to reverse this. The effects on arrhythmogenesis remain unclear, however, and therefore the effects of chronic DVA on dofetilide-induced arrhythmias and the reversibility with chronic CRT were studied.

Methods: In anesthetized dogs, DVA was created by left bundle branch block or right ventricular pacing (VDD mode using 2 leads), after creation of AV-block. CRT was established by atrioventricular pacing. Dofetilide (0.025mg/kg/5min) was administered under bradycardia (VVI mode, 60/min), directly after creation of DVA, after 4 weeks DVA and after 8 weeks CRT. Primary outcome was quantified by combining the number of ectopic beats, short runs of ectopic beats, episodes of TdP and defibrillations in a single arrhythmia score. Besides regular electrophysiological measurements, including short-term variability (STV), the time after dofetilide required to obtain 25 ms-increase of left ventricular monophasic action potential duration (T25) was calculated. Arrhythmia score and T25 were validated before.

Results: QRS was significantly widened by DVA (from 75±4 to 121±10 ms, $P<0.001$) and partly restored by CRT (to 96±10 ms, $P<0.01$). Serial tests revealed that DVA increased arrhythmia score from 2±3 to 17±19 ($P<0.05$, n=9), whereas CRT returned arrhythmia score from 23±19 to 5±6 ($P<0.05$, n=8). These effects were neither linked to differences in repolarization duration nor STV, but T25 was modified (from 2.1±0.3 to 1.7±0.2 minutes; $P<0.01$) and an increase from 1.8±0.4 to 2.2±0.4 minutes (P=NS) after CRT.

Conclusion: Chronic DVA without bradycardia increases susceptibility to dofetilide-induced arrhythmias by creation of electrical remodeling and this arrhythmogenesis is reversed after chronic CRT.

Keywords: Artificial cardiac pacing; Bundle-branch block; Remodeling; Resynchronization therapy; Torsade de Pointes; Variability of repolarization; Ventricular arrhythmia

INTRODUCTION

Left bundle branch block (LBBB) and right ventricular (RV) apical pacing are both known causes of electrically dyssynchronous left ventricular activation (DVA). DVA causes dyssynchronous left ventricular (LV) contraction and relaxation related to early-systolic shortening of the early-activated, anteroseptal region and pre-stretching of the late-activated, posterolateral part of the LV. DVA decreases LV pump function, induces ventricular remodeling and is associated with negative outcomes, including development of heart failure, atrial fibrillation, stroke and probably also an increased mortality rate.[1-6] Also animal models have been developed to study the isolated effects of DVA; especially the dog is a suitable species, in which the activation patterns and mechanical consequences of LBBB and RV pacing have been studied in detail.[7-11]

In patients with DVA, cardiac resynchronization therapy (CRT) is a therapy aimed at restoring mechanical synchrony to improve LV pump function and to reverse the ventricular remodeling. CRT is usually performed by biventricular stimulation with a conventional right ventricular apical (or septal) electrode and a second electrode implanted epicardially via the coronary sinus at the late-activated, posterolateral free wall of the LV. The benefit of CRT has been shown in several studies, especially in patients with impaired LV function (ejection fraction ≤35%) and LBBB (class I recommendation).[12] The same holds true for DVA caused by RV apical pacing, although the evidence is less strong.[12] The beneficial effect of chronic CRT was also demonstrated in the canine model of isolated LBBB: the 'dyssynchronopathy' caused by eight weeks of LBBB was successfully reversed.[8]

Patients with heart failure not only suffer from mechanical dysfunction but are also at an increased risk of sudden arrhythmic death. The latter is associated with persistent changes of the electrical properties of the ventricular myocardium, so-called ventricular electrical remodeling, which seems to be worsened by DVA and improved by CRT (this is reviewed by Aiba et al.[13]). Altered activation in normal hearts is known to cause ventricular electrical remodeling as well, with changes in T wave that (temporarily) persist after restoration of normally conducted sinus rhythm (therefore called 'cardiac memory').[14] Yet, it is still uncertain whether this electrical remodeling increases susceptibility to Torsade de Pointes (TdP) arrhythmias and whether this is reversible with CRT. Aim of this study was to investigate the effects of chronic isolated DVA in dogs on susceptibility to (dofetilide-induced) TdP arrhythmias and the reversibility with CRT. Secondary objective was to link the arrhythmogenic outcome to electrophysiological parameters that are used to quantify electrical remodeling, both before and after dofetilide administration.

METHODS

Animal handling, electrophysiological recordings and test for arrhythmia susceptibility

All experiments were approved by the committee for experiments on animals of Utrecht University, the Netherlands and animal handling was in accordance with the Dutch Law on Animal Experimentation and the European Directive for the Protection of Vertebrate Animals Used for

Experimental and Other Scientific Purposes (European Union Directive 86/609/EEC). In awake conditions, a standard 6-lead surface ECG was recorded at sinus rhythm to exclude pre-existent significant ECG abnormalities. Dogs were housed in pairs, if possible and food pallets and water were provided ad libitum. Pacemaker functioning and ECG were monitored two to three times per week.

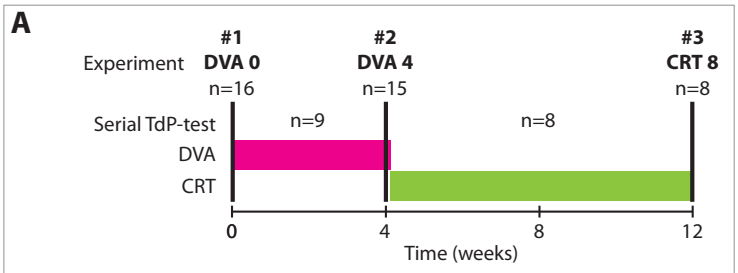
Study measurements were performed in three serial experiments (*Figure 1*) under general anesthesia, induced with sodium pentobarbital (25 mg/kg i.v.) and maintained with isoflurane 1.5% in 1:2 O_2:N_2O. Sixteen dogs were included (Marshall, USA; body weight 20±2.4 kg, age 1.1±0.2 years, four males) for these experiments. Perioperative care is described in more detail in the supplement. A 6-lead surface ECG and four precordial leads (LL, LU, RL, V10) were recorded, whereas monophasic action potentials (MAP) were recorded at the free wall of the LV (usually at the apical 1/3 of the heart), and at the free wall of the RV (more variable sites). Before administration of dofetilide, 15 minutes baseline were recorded during pacing in VVI mode at 60/min (or 70/min if required; then also during the other serial experiments). Dofetilide (0.025 mg/kg/5min) was dissolved in 0.1 ml 0.1 M HCl and diluted in a volume of about 10 ml NaCl 0.9% and infused with a syringe pump. If TdP occurred the infusion was aborted. Cardioversion (10J-150J-200J biphasic) was performed if an arrhythmia did not terminate within 10 s. A 15 min observation period was used to determine arrhythmia score.

Experiment 1, part 1: Establishing DVA activation

The used leads, pulse generators and implantation procedures (in some dogs in an additional, preparatory experiment) are described in the supplemental methods. If DVA was successfully created, the dogs were included (*Figure 1*). In the original study design AV-block was created, while DVA was established by the combination of LBBB and RV high-septal pacing (preferably direct His-bundle pacing) (group 2 (n=4) in *Figure 1B*). If creation of DVA was unsuccessful, dogs were either removed from the study or used in a group in which DVA was created by isolated LBBB without pacing and without AV-block (Group 1 (n=4) in *Figure 1B*). Finally, a third group was added, in which DVA was created by AV-block and RV apical pacing (n=8).

Experiment 1, part 2: Study measurements at acute DVA

Both before and directly (usually within 30 minutes) after creation of DVA, electrophysiological measurements were performed at sinus rate in 15 of 16 dogs. After creation of AV-block (groups 2 and 3) electrophysiological parameters were recorded as described below and dofetilide was administered to test arrhythmia susceptibility. These dogs were excluded from the serial analysis that compared acute and chronic DVA, because arrhythmia susceptibility could not be studied acutely after DVA. After the experiment, the pacemaker (groups 2 and 3) was programmed to VDD mode (or DDD mode with lower and upper tracking rate programmed at their extremes, which were typically 30 and 190 bpm).



B

	Group 1	Group 2	Group 3	Total, n
n	4	4	8	16
DVA characteristics				
LBBB	+	+	-	8
RV high-septal pacing	-	+	-	4
RV apical pacing	-	-	+	8
AV-block	-*	+	+	8
TdP-tests (dofetilide)				
Exp. #1 (DVA 0), n	0	3	8	11
Exp. #2 (DVA 4), n	4	4	6	14
Exp. #3 (CRT 8), n	2	3	3	8
Serial DVA 0 & 4, n	0	3	6	9
Serial DVA 4 & CRT 8, n	2	3	3	8
Serial all 3 exp., n	0	2	3	5

Figure 1. Overview of the serial experiments used for electrophysiological measurements

The flowchart shows the three serial experiments used for electrophysiological measurements, performed under general anesthesia (panel A). DVA was created in three ways (panel B), by either left bundle branch block ablation (LBBB; n=4, group 1), LBBB followed by AV node ablation and His-bundle/high-septal pacing from the right ventricle (RV) (n=4, group 2) or AV-block ablation followed by RV apex pacing (n=8, group 3). DVA was maintained for four weeks, either as unpaced sinus rhythm with LBBB (group 1) or by VDD mode pacing (groups 2 and 3). After the experiment at t = 4 weeks, the activation was changed to biventricular VDD pacing (cardiac resynchronization therapy; CRT) and maintained for eight weeks.

In most experiments dofetilide could be administered to determine arrhythmogenic outcome (primary purpose) and to link the outcome to the electrophysiological surrogate parameters (secondary purpose). These experiments were performed during bradycardia by pacing at a fixed cycle length of 1000ms (60 bpm; or 857 ms = 70 bpm) and therefore required AV-block. Please note that a number of dogs were lost to follow-up, due to perioperative complications (n=4), malfunctioning of the CRT-system (n=2) or inclusion for a pilot study in which CRT was performed after four weeks of additional bradycardic DVA activation (n=2 dogs). Please also note that in the figure, the implantation of the leads and devices, in some dogs performed in a separate experiment, are not shown.

Experiment 2: Study measurements at chronic DVA

After four weeks of DVA activation, the same measurements were repeated at acute DVA in group 2 and 3, whereas in group 1 first AV-block was created followed by maintenance of DVA activation by RV apical pacing. After the experiment, including TdP susceptibility test, the CRT device was used to start biventricular pacing in DDD mode, with PQ similar to sinus rhythm (typical sensed AV-delay 100 ms), a VV-delay of 0 ms and lower and upper tracking rate at their extremes (30 and 210/min).

Experiment 3: Electrophysiology after chronic CRT and termination

After eight weeks CRT, a similar experiment under general anesthesia was performed, but with maintenance of the CRT activation pattern at the slow heart rate. At the end of this experiment dogs were euthanized by excision of the heart.

Data analysis

Surface ECG was recorded and analyzed with EPTracer (CardioTek, Maastricht, Netherlands). The measurements were performed off-line, manually using calipers in lead II. The mean of at least five beats was calculated. QRS was measured from pacing stimulus artefact if present (exception: direct His-bundle pacing). QT was measured till the end of the T wave in lead II and heart rate correction was applied using Van de Water's formula ($QTc = QT - 0.087 \cdot (RR - 1000)$).[15] JT was calculated by subtracting QRS from QT. Tpeak-end was measured in lead II, from the peak of the T wave (or nadir if the T wave was negative of biphasic), to the end of the T wave as a measure of global dispersion of repolarization.[16] LV and RV activation time were defined as the interval from QRS onset to the steepest upstroke of the LV MAP and RV MAP, respectively, and the difference was calculated as a measure of interventricular dispersion of activation, with a positive value indicating later LV than RV activation. MAP duration was measured semi-automatically, at 80% repolarization, using custom-written software in Matlab (Mathworks, Natick, USA). Beat-to-beat variability of repolarization was quantified as short-term variability of LV MAP duration (STV). STV is calculated as follows: the absolute difference of two consecutive beats is divided by the square root of 2 and STV is calculated by averaging 30 (consecutive) values. In addition, using the same calculation, STV_{RV} was measured from the RV MAP.

Single ectopic beats were defined as ectopic beats initiated before the end of T wave and TdP as a run of at least five of such ectopic beats, with polymorphic twisting of the QRS-axis on the surface ECG. Measurements after dofetilide were performed at t=5min or in the last available 30-beats window before onset of arrhythmogenesis (ectopic beats). Arrhythmia score, which is based on the number of beats of the three most severe arrhythmias, was calculated as follows: each regularly paced beat within 15 min after start of dofetilide is scored 1 + x, where x equals the number of polymorphic ectopic beats, initiated within the T wave of the preceding beat, with a maximum of 50 and arbitrary value of 50, 75 or 100, for 1, 2 or ≥3 cardioversions, respectively;

arrhythmia score is the mean of the three highest scores and, thus, can range from 1 (if no ectopic beats are observed) to 100 (if three arrhythmias are induced that require each three or more cardioversions) [see also *Chapter 6*]. T25 was defined as the time from start of dofetilide required to obtain a 25ms-increase of LV MAPD and calculated by fitting an exponential curve over the interval from t=0 to t=2 min. The interval was extended 30 s after the estimated T25 if T25 was not reached within 2 min to avoid extrapolation and T25 was only calculated if more than 1 min was available. We have validated T25 and arrhythmia score before by comparing these parameters in similar studies in dogs, in which differences in arrhythmogenic outcome were caused by differences in bradycardia and activation pattern [*Chapter 6*].[17]

To limit the influence of ectopic beats and RR variation, ectopic beats and the two beats thereafter were excluded. MAP recordings were only used for analysis if signal quality was considered acceptable (stable morphology, plateau amplitude ≥10 mV).

Statistical analysis

All values are expressed as mean±SD, unless stated otherwise. Statistical analysis was performed with the software R (R Foundation for Statistical Computing, Vienna, Austria). Two-tailed tests were used and a P value <0.05 was considered significant, except for electrophysiological parameters: due to the number of parameters and the two measurements, before and after dofetilide administration, here a P<0.01 was considered significant, whereas a P value <0.05 was considered a trend. Serial analyses were performed with paired student's t tests. Where appropriate, logarithmic transformation was applied or a nonparametric, Wilcoxon signed ranks test was used.

RESULTS

Acute electrophysiological effects of DVA creation

The acute effects of DVA were studied at intrinsic sinus rate (*Table 1*). QRS increased after creation of DVA (P<0.001). The LV activation time (of the MAP) was severely delayed (P<0.001) with only a small (trend to) a delay of RV activation, resulting in a net large interventricular difference in activation time (ΔAT in *Table 1*; P<0.001). The increase of QRS by DVA was associated with an increase of QT, whereas JT and LV MAPD actually decreased by about 10 ms. RV MAPD did not decrease, resulting in a decrease of interventricular dispersion of repolarization duration (ΔMAPD in *Table 1*). However, this parameter does not account for increase of LV activation time, which was larger than the decrease of LV MAPD. This may increase global dispersion of (endocardial) repolarization times, also based on the significant increase of $T_{peak\text{-}end}$ (P<0.001). STV was not influenced by the creation of DVA.

Table 1. Acute effects of DVA on electrophysiological parameters, at sinus rate

	NSR	DVA	P value
RR, ms	565 ± 55	548 ± 51	0.051
QRS, ms	75 ± 4	121 ± 10	<0.001†
LV AT, ms	14 ± 4	60 ± 14	<0.001†
RV AT, ms	17 ± 8	25 ± 11	0.046
ΔAT, ms	-4 ± 9	37 ± 16	<0.001†
QT, ms	279 ± 13	314 ± 20	<0.001†
QTc, ms	316 ± 11	354 ± 19	<0.001†
JT, ms	204 ± 14	194 ± 16	0.005*
$T_{peak-end}$, ms	58 ± 11	76 ± 11	<0.001†
LV MAPD, ms	211 ± 11	199 ± 14	<0.001†
RV MAPD, ms	192 ± 14	193 ± 12	0.687
ΔMAPD, ms	18 ± 10	5 ± 17	0.005*
STV, median [IQR], ms	0.3 [0.2 - 0.4]	0.3 [0.3 - 0.3]	0.295
STV_{RV}, median [IQR], ms	0.4 [0.3 - 0.4]	0.3 [0.3 - 0.5]	0.599

Data are mean±SD (or median with interquartile range) obtained from 15 dogs in which DVA acutely (usually within 30 min after initiation) was compared to the normally conducted sinus rhythm (NSR) before initiation. LV, left ventricular; AT, activation time; RV, right ventricular; ΔAT, LV AT – RV AT; $T_{peak-end}$, T wave peak to end interval; MAPD, monophasic action potential duration; ΔMAPD, LV MAPD minus RV MAPD; STV, short-term variability of LV MAPD; STV_{RV}, short-term variability of RV MAPD. Serial measurements of MAPD was available in n=14 (LV) and n=12 (RV). *, P<0.01; †P<0.001.

Effects of chronic DVA on electrophysiological parameters at baseline

The electrophysiological parameters after four weeks of DVA were compared with the serial measurements acutely after DVA creation, under similar conditions: DVA activation at a paced heart rate of 60 bpm. QRS and activation times remained the same but also repolarization duration parameters measured at baseline (before dofetilide) were not changed significantly, although RV MAPD showed a trend to an increase (P=0.047; *Table 2*). The parameters reflecting spatial dispersion were neither changed. Beat-to-beat variability of repolarization showed a clear trend to a small increase (P=0.026).

Effects of DVA on dofetilide-induced arrhythmogenesis

Acutely after creation of DVA, dofetilide was administered in eleven animals and this resulted in an AS of 5.1±10 (median [interquartile range]: 1.3 [1.3-1.7]). In two dogs, repetitive TdP episodes were induced and unfortunately, one of these dogs died due to the TdP/ventricular fibrillation.

Primary outcome was the serial analysis of arrhythmogenic outcome before and after remodeling due to chronic DVA (n=9 dogs, see also *Figure 1*): arrhythmia score significantly increased from 2.2±2.7 to 17±19 (median [interquartile range]: 1.3 [1.3-1.3] and 3.3 [2.0-37], respectively); P=0.021; *Figure 2*). An individual ECG example of observed arrhythmogenesis after four weeks DVA is shown in the upper part of *Figure 3*.

As observed in baseline, after dofetilide repolarization parameters were not different, except for T25, which could only be measured after dofetilide and was significantly shortened at chronic

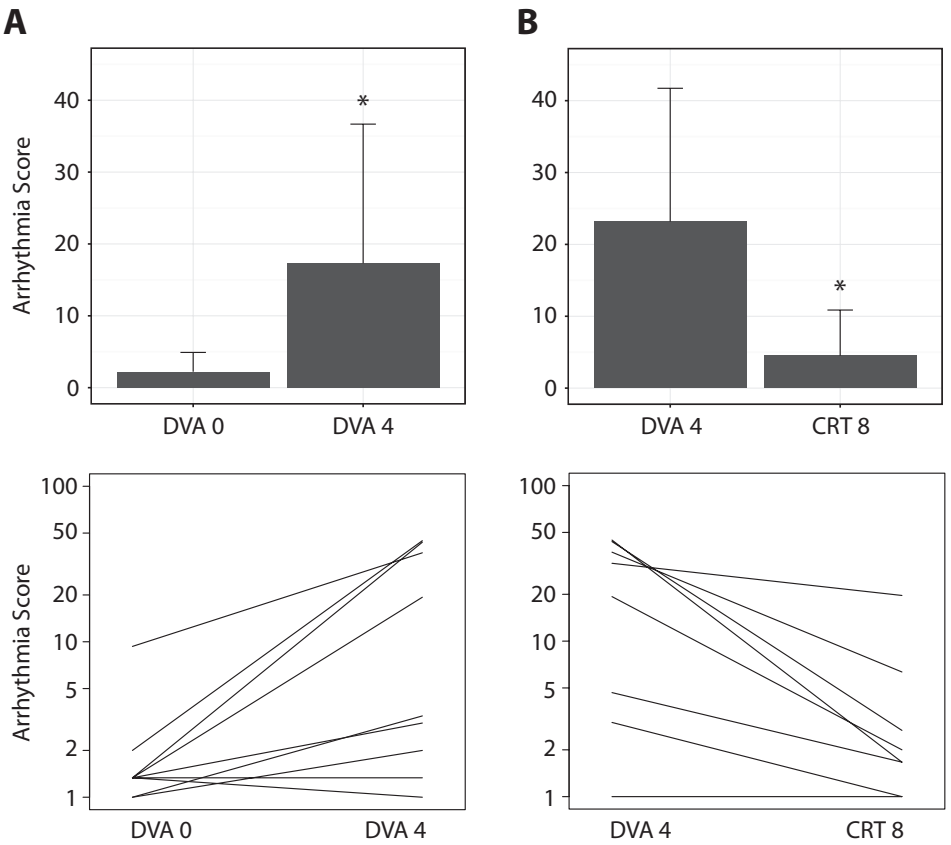


Figure 2. Effects of chronic DVA and CRT on arrhythmogenesis

A: The effect of chronic dyssynchronous ventricular activation (DVA) on arrhythmia score after dofetilide administration, at acute and chronic (4 weeks) DVA (n=9).

B: Anti-arrhythmic effect of 8 weeks cardiac resynchronization therapy (CRT 8) in dogs with chronic DVA (n=8). In the bar plots, data are summarized as mean±SD (*, P<0.05). The line charts show the data of the individual dogs, on a logarithmic scale. Only four dogs showed a high arrhythmia score after DVA and there seemed to be a correlation with the arrhythmia score at acute DVA. After CRT, reversibility was seen in most dogs, although especially in one dog, a lack of benefit could be observed: a high arrhythmia score was maintained after CRT.

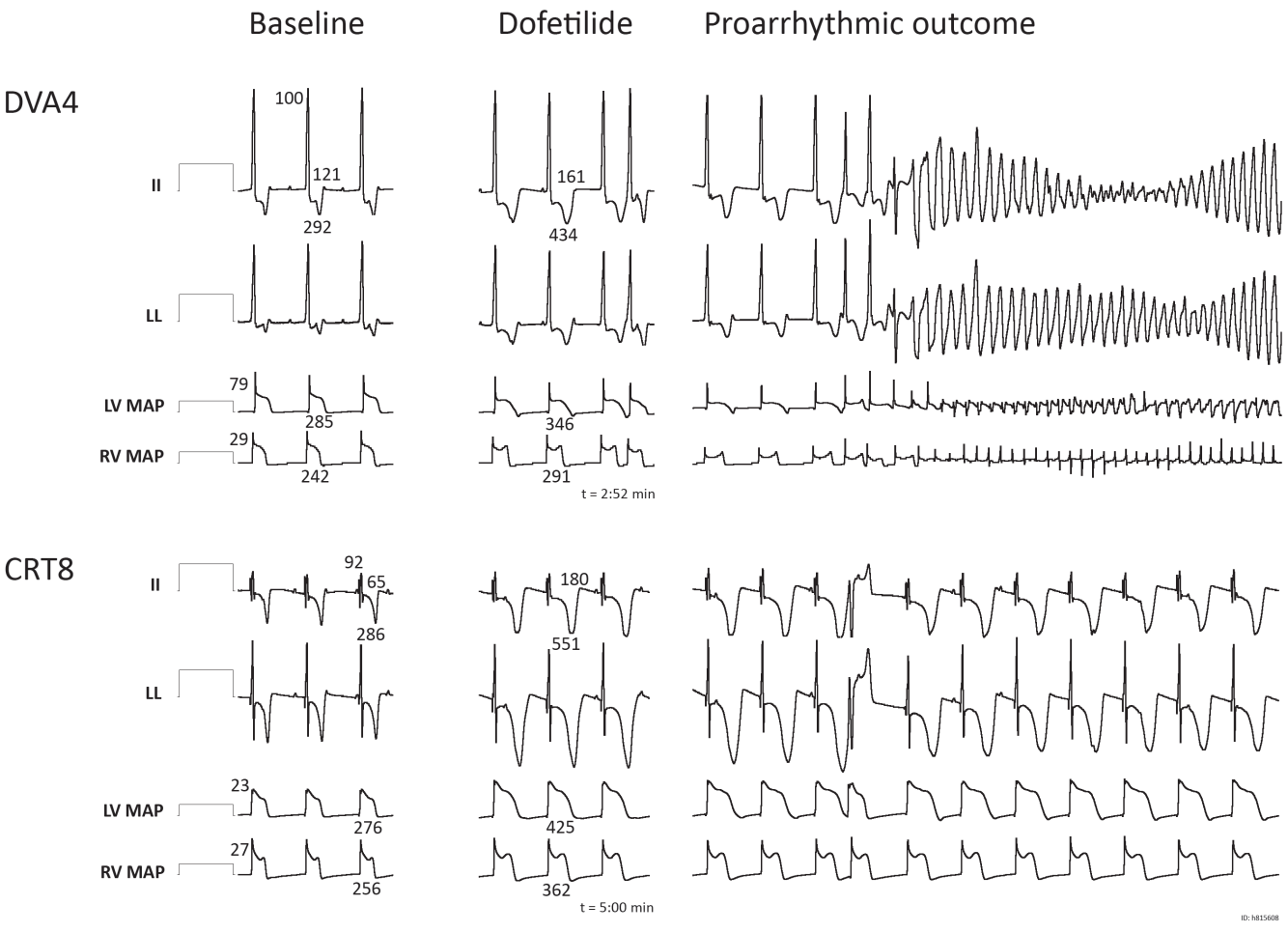

Baseline
Dofetilide
Proarrhythmic outcome
DVA4
100
121
292
II
LL
79
285
LV MAP
29
242
RV MAP
161
434
346
291
t = 2:52 min
CRT8
92
65
286
180
551
23
276
425
27
256
362
t = 5:00 min
ID: h815608

Figure 3 (Left). ECG example of the arrhythmogenesis after chronic DVA and CRT

This figure shows the surface electrocardiogram (ECG) and monophasic action potentials (MAP) in a dog after four weeks DVA (upper panels) and after eight weeks CRT. Shown are ECG lead II, the precordial lead LL and the left (LV) and right ventricular (RV) MAP. In this dog dyssynchronous left ventricular activation (DVA) was created by the combination of left bundle branch block and AV-block with his-bundle pacing (dog from group 2 in *Figure 1B*). At acute DVA, no TdP episodes were observed but at four weeks, a single TdP episode was observed after dofetilide (right panel), with an arrhythmia score of 19.3, whereas after eight weeks CRT only single ectopic beats were observed (arrhythmia score of 2.0).

The most left panels show baseline during pacing at 1000 ms cycle length, before administration of dofetilide. Values are, from top to bottom, QRS, $T_{peak\text{-}end}$, QT, LV activation time, LV MAP duration, RV activation time and RV MAP duration. The middle panel shows the repolarization parameters after dofetilide (before the first ectopic beat). After CRT, the first ectopic beat occurred later and therefore repolarization parameters could prolong more than during four weeks DVA.

DVA (P=0.002; *Table 2*). The individual tracings of the action potential increase over time and the estimation of T25 are shown in *Figure 4A*. The apparently reduced repolarization reserve was linked to earlier onset of arrhythmogenesis: measurements (performed at 5 min or before onset of arrhythmogenesis) were performed significantly earlier after start of dofetilide infusion, than at acute DVA (at 2.8±0.7 min vs. at 4.3±1.2 min, P<0.001).

Effects of chronic CRT on electrophysiological parameters at baseline

Compared with the baseline situation of chronic DVA, CRT partly restored QRS duration (P=0.006; *Table 3*) and this was associated with a trend to reduction of QT (P=0.040), without alteration of JT (P=0.96) or other repolarization parameters, including STV (P=0.21), although the mean of 0.4±0.2 ms was similar to the STV during acute DVA.

Effects of chronic CRT on dofetilide-induced arrhythmogenesis

Serial comparison with the experiments before start of CRT (i.e. chronic DVA) revealed that arrhythmia score after dofetilide administration was significantly reduced, from 23±19 (median [interquartile range] 26 [4.3-39]) to 5±6 (median 1.8 [1.5-3.6]), P=0.022; *Figure 2 and 3*). Although T25 at chronic CRT was the same as in unremodeled circumstances, statistically the increase was neither significant nor a trend (P=0.14; *Table 3 and Figure 4B*). Due to delayed onset of ectopic beats, the measurements were performed significantly later after start of dofetilide infusion (4.9±0.4 min vs. 3.1±1.1 min, P=0.002) and the values of LV and RV MAPD could reach higher values.

Table2. Electrophysiological effects of chronic DVA

	DVA 0 weeks	DVA 4 weeks	P value
Baseline			
QRS, ms	118 ± 11	118 ± 12	0.948
LV AT, ms	51 ± 9	59 ± 13	0.064
RV AT, ms	25 ± 14	29 ± 11	0.276
ΔAT, ms	26 ± 12	30 ± 15	0.272
QT, ms	374 ± 17	380 ± 18	0.476
JT, ms	257 ± 13	262 ± 20	0.552
$T_{peak-end}$, ms	94 ± 20	94 ± 20	0.933
LV MAPD, ms	253 ± 17	257 ± 28	0.656
RV MAPD, ms	225 ± 14	242 ± 26	0.047
ΔMAPD, ms	28 ± 18	15 ± 24	0.159
STV, median [IQR], ms	0.3 [0.3 - 0.5]	0.7 [0.6 - 0.9]	0.026
STV_{RV}, median [IQR], ms	0.4 [0.3 - 0.7]	0.6 [0.3 - 1.0]	0.207
Dofetilide			
QT, ms	510 ± 77	543 ± 59	0.234
JT, ms	392 ± 79	425 ± 53	0.209
$T_{peak-end}$, ms	157 ± 53	181 ± 61	0.243
LV MAPD, ms	356 ± 73	343 ± 60	0.682
RV MAPD, ms	296 ± 33	296 ± 43	0.969
STV, median [IQR], ms	0.9 [0.8 - 1.2]	1.9 [1.4 - 2.3]	0.306
STVRV, median [IQR], ms	0.8 [0.7 - 1.1]	1.0 [0.6 - 1.1]	0.729
T25, minutes	2.1 ± 0.3	1.7 ± 0.2	0.002*

Values are mean±SD (or median with interquartile range) obtained from dogs in which electrophysiological measurements at baseline and after administration of dofetilide, were tested both acutely after creation of dyssynchronous ventricular activation (DVA) and after four weeks remodeling due to DVA at sinus rate (n=9). Both measurements were performed during the same paced heart rate (VVI 60/min). Serial measurements of MAPD were available in n=9 (after dofetilide n=8).

LV, left ventricular; RV, right ventricular; AT, activation time; ΔAT, difference between LV and RV AT; $T_{peak-end}$, T-peak-to-end interval; MAPD, monophasic action potential duration; ΔMAPD, difference between LV and RV MAPD; STV, short-term-variability of LV MAPD; T25, dofetilide infusion time required to obtain 25ms-increase of LV MAPD. *, P<0.01 vs. DVA 0 weeks.

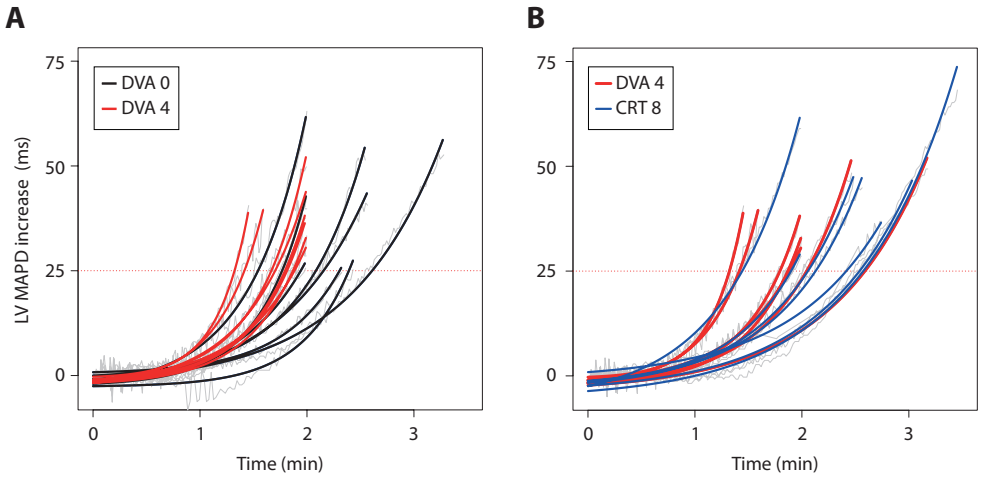


Figure 4. Initial increase of left ventricular monophasic action potential duration after dofetilide

The figure shows the initial increase of the left ventricular monophasic action potential duration (LV MAPD) after start of dofetilide for individual dogs (grey lines) and the exponential fits (thick, smooth, lines). The latter were used for calculation of the time required to obtain 25 ms-increase of LV MAPD (T25). This (somewhat arbitrarily chosen) 25ms-threshold is shown in the figure as the red line.

A, acute (0 weeks; black) and chronic (4 weeks; red) DVA. After remodeling due to DVA a faster initial increase of LV MAPD is observed and T25 values were significantly shorter (n=8; Table 2).

B, chronic DVA (red) and chronic CRT (8 weeks; blue). Although after CRT the T25 values became similar to acute DVA, the number of dogs is smaller and more variation is observed (also in the dogs with chronic DVA), resulting in absence of significance or trend in T25 value (n=7; Table 2).

DISCUSSION

Main finding of the study is that isolated chronic DVA increases susceptibility to (dofetilide-induced) TdP arrhythmias, which is reversible with CRT. The commonly used repolarization parameters were unable to provide evidence for this electrical remodeling in baseline or after dofetilide, with the exception of the novel parameter T25.

Relevance of altered activation for torsadogenic remodeling in dogs

The design of the study was inspired on previous studies in dogs with chronic, complete AV-block. This model is used to specifically study acquired long QT/TdP and is characterized by a high TdP susceptibility. The torsadogenic risk in the model can be extrapolated to humans: drugs with known torsadogenic potential usually result in a high incidence of TdP, whereas drugs with low or no risk of TdP were safe in the model, even if prolonging QT. Dofetilide is used as 'gold standard' (positive control) in the model and causes TdP in about 75% of the dogs, regardless

Table 3. Comparison of electrophysiological parameters before and after chronic CRT

	DVA 4 weeks	+ 8 weeks CRT	P value
Baseline			
QRS, ms	115 ± 14	96 ± 10	0.006*
LV AT, ms	60 ± 12	45 ± 13	0.033
RV AT, ms	26 ± 14	30 ± 9	0.445
ΔAT, ms	35 ± 17	15 ± 11	0.038
QT, ms	376 ± 21	356 ± 39	0.040
JT, ms	261 ± 17	261 ± 41	0.964
$T_{peak-end}$, ms	95 ± 19	70 ± 21	0.095
LV MAPD, ms	247 ± 30	246 ± 15	0.971
RV MAPD, ms	229 ± 13	234 ± 12	0.396
ΔMAPD, ms	18 ± 20	12 ± 11	0.514
STV, median [IQR], ms	0.6 [0.3 - 1.1]	0.4 [0.3 - 0.5]	0.205
STV_{RV}, median [IQR], ms	0.4 [0.3 - 0.8]	0.4 [0.4 - 0.4]	0.400
Dofetilide			
QT, ms	515 ± 38	535 ± 59	0.308
JT, ms	400 ± 33	440 ± 63	0.060
$T_{peak-end}$, ms	179 ± 39	143 ± 29	0.071
LV MAPD, ms	317 ± 34	376 ± 35	0.001*
RV MAPD, ms	281 ± 17	331 ± 29	0.007*
STV, median [IQR] , ms	1.4 [1.3 - 2.0]	1.3 [1.0 - 1.4]	0.114
STV_{RV}, median [IQR], ms	1.0 [1.0 - 1.4]	0.9 [0.7 - 1.6]	0.831
T25, minutes	1.8 ± 0.4	2.2 ± 0.4	0.135

Values are mean±SD, or median with interquartile range (IQR), from eight dogs that were tested serially (including dofetilide administration), before and after chronic treatment with cardiac resynchronization therapy (CRT). Measurements were performed during a paced heart rate (VVI 60/min). Serial measurements of MAPD were available in n=7.

Abbreviations are explained in *Table 1*. *, P<0.01 (CRT 8 weeks vs. DVA 4 weeks).

of whether the susceptibility challenge is performed during idioventricular rhythm or during a 1000 ms paced cycle length.[17-19]

AV-block creation results in bradycardia, AV-dyssynchrony and an altered ventricular activation pattern due to uncontrolled idioventricular rhythm. Originally, the bradycardia (about 50% reduction of heart rate) was considered the most important factor, but a role for ventricular activation pattern was suggested more recently: in AV-blocked dogs, induction of bradycardia with maintenance of a more physiological activation pattern than during idioventricular rhythm (by high-septal pacing at lowest captured rate) resulted in a decreased TdP incidence at 1000 ms paced cycle length, of 30%.[17] The combination of bradycardia and DVA (by RV apical pacing

at lowest capture rate after creation of AV-block) resulted in repetitive dofetilide-induced TdP episodes in 6 of 8 (75%) dogs at the slow heart rate, which is similar to the TdP incidence in dogs with remodeling due to chronic idioventricular rhythm.[17]

In the present study, we did not have a component of bradycardia to induce remodeling during the phase of chronic DVA and CRT. Arrhythmia susceptibility, however, was once again, studied at a paced cycle length of 1000 ms, because bradycardia is required for TdP induction in the model,[20] but also to improve comparability in the serial experiments and with other studies.

Arrhythmic outcome: isolated DVA is pro-arrhythmic

The effects of chronic, isolated DVA on electrophysiology and arrhythmogenesis have been scarcely investigated and so far not in vivo. Based on in vitro analysis, it has been suggested that isolated LBBB in dogs induces proarrhythmic remodeling, without actual different arrhythmogenic endpoints.[21] Wedge preparations from the early-activated, anterior myocardium of the LV and from the late activated lateral myocardium of chronic LBBB hearts were compared. The APD was significantly shorter in the late than in the early activated myocardium, although the absolute APDs were not significantly different from the controls. Also conduction velocity and refractory period were reduced in the late activated myocardium, but this was not associated with enhanced arrhythmogenesis. Limitations of this study include that transmural dispersion could not be measured, and that the electrophysiological measurements were limited to the small wedge preparations, which might hinder translation to the whole heart, in vivo animals and humans.[22]

We found that isolated chronic DVA significantly increased arrhythmia score. The reached value, of 17±19 (mean±SD), was similar to the arrhythmia score in a previous study in which bradycardia was the dominant factor for remodeling (14±22) [*Chapter 6*].[17] This suggests that isolated chronic bradycardia and isolated chronic DVA have a roughly similar proarrhythmic effect, whereas the combination seems to result in a higher arrhythmia score (28±24) [*Chapter 6*].[17] It was necessary to use arrhythmia score, because at first, an a-priori calculated sample size was estimated at n=18 if the dichotomous outcome, TdP incidence (≥3 TdP episodes in 10-15 min) would be used in serial measurements (this is the classical parameter that has been used in many previous publications). In addition, at acute DVA, paced at 1000 ms cycle length, dofetilide induced TdP in 2 of 11 dogs (18%). This was higher than expected, because induction of ≥3 TdPs has not been observed before in acute AV-block dogs under similar anesthesia. On the other hand, multiple ectopic beats and incidental TdP episodes have been observed and it was shown that arrhythmia score after dofetilide was significantly higher than the score during baseline.[17]

Arrhythmic outcome: CRT is anti-arrhythmic

In patients, CRT has anti-arrhythmic properties that are related to the biventricular pacing.[23] This anti-arrhythmic effect has been linked to structural reverse remodeling after chronic CRT.[24] In contrary, also proarrhythmic effects of CRT have been reported, especially during the acute phase. The induction of arrhythmias may be related to repolarization because QT prolongation

and TdP have been observed.[25-27] Explanation may be the nonphysiological LV stimulation from epicardium to endocardium.[28,29]

In a study, that was performed at the same institution as the previously mentioned study of Spragg et al., three weeks of tachypaced DVA was followed by either three more weeks of tachypaced DVA or three weeks of tachypaced CRT.[30] At t=6 weeks, isolated myocytes from the lateral LV in the CRT group showed shorter action potentials than in the group without CRT, linked to a reduction of early afterdepolarization frequency. Both groups displayed heart failure. Because myocytes isolated from the anterior LV wall did not show a difference in action potential duration, the authors also concluded that the substrate for arrhythmias was reduced, based on a decrease of the regional dispersion within the LV after CRT. No in vivo data have been presented. If specifically interested in DVA, this study has several limitations: (1) it remains unknown whether the differences versus control were caused by the tachypacing or by the DVA (because the control group was not (atrially) tachypaced; (2) whether the effect at six weeks was caused by reverse remodeling in the CRT group or by progression of remodeling in the DVA group; (3) the effects on arrhythmogenesis could only be based on surrogates (action potential duration, dispersion and EADs), differences were found especially at much lower heart rates than in vivo (actually even unphysiological long cycle lengths of 2000-4000 ms).

We observed a significant anti-arrhythmic effect of CRT in this study on dogs with only a history of isolated DVA. Although not observed, we cannot exclude that a proarrhythmic effect may be present in a minority of dogs (we did observe that one dog did clearly not benefit; *Figure 2B*). We did not study the acute effects of CRT on arrhythmogenesis, like we did for DVA, and therefore we cannot exclude that a remodeling-independent (acute) effect contributes to the observed anti-arrhythmic effects of CRT.

Surrogate electrophysiological parameters in baseline or after dofetilide

We failed to detect an increase of repolarization duration from the surface ECG or MAPs. The LV MAP measurements were typically performed at the apical 1/3 of the free wall of the LV. Although these MAP measurements may not have been located at the latest activated myocardium, we did find a decreased T25, which implies that electrical remodeling was present at, or near the site of measurement. With the knowledge that DVA did not result in changes in repolarization duration, the lack of differences after chronic CRT (*Table 3*) was anticipated for these parameters. Although the T25 after chronic CRT was similar as at acute DVA, we did not find a trend towards significance. However, this study lacked power to detect improvement of T25 (a post hoc sample size calculation based on the observed values and a power level of 80%, resulted in a sample size of n=22).

Several studies have suggested that beat-to-beat variability of repolarization is superior to repolarization duration itself as risk marker for TdP.[31-33] In the current study, we did find a modest association of the proarrhythmic remodeling with STV (*Table 2*). Moreover, in the earlier mentioned study (see *Chapter 6*) in which DVA was combined with bradycardia, no trend to increase

of STV was observed despite the high arrhythmia susceptibility.[17] Therefore we conclude that, like repolarization duration, STV is not useful for quantification of electrical remodeling and TdP-risk in dogs with DVA, with or without chronic bradycardia. Further research is required to elucidate the precise role of the activation sequence.

Site of electrical remodeling in relation to arrhythmogenesis: conflicting data

Ventricular electrical remodeling seems to be important for acquired TdP susceptibility and this is typically hallmarked by action potential prolongation and an increased susceptibility to triggered activity. Based on the published literature, during DVA, especially the late-activated region of the left ventricle seems to remodel, related to the increased strain. However electrophysiological changes also occur at the sites of early activation.[13,21,34,35] In another recent study in minipigs, remodeling due to altered activation was not related to a significant prolongation of parameters representing repolarization.[36] AV-block creation was followed by one year of epicardial LV apical pacing or RV free wall pacing. Compared to unpaced controls, QT intervals were roughly 5% longer, but statistically this was neither significant nor a trend. Action potentials from isolated LV free wall myocytes had a significantly shorter duration in both paced groups. Although similarities with the study of Spragg et al. were also observed, another difference was that the activation time (early versus late) seemed to be of less importance for the found results. Also in other studies altered ventricular activation did not or only slightly influence QT, including in cardiac memory induced by chronic LV epicardial pacing in dogs,[16] RV paced rabbits, in which only addition of bradycardia was able to induce electrical remodeling[37] and chronic, complete AV-block dogs in which reversibility by RV apex pacing (n=7) or CRT pacing (n=6) was studied.[38] In the latter study, repolarization became prolonged by chronic idioventricular rhythm but this was not reversible. However, in all these studies the link with arrhythmia susceptibility remained unknown. Interestingly, arrhythmogenic outcome was included in a study conducted by Winckels et al. that was aimed at analysis of reversibility of bradycardia-induced remodeling. After three weeks AV-block + high-septal pacing at lowest captured rate, a physiological heart rate was restored by mode switching to VDD. Although, electrophysiological parameters improved, after dofetilide unmasking was observed with similarly high arrhythmogenic outcome.[39]

Also in a previous study in which DVA was combined with chronic bradycardia we found a similar pattern, including absence of LV MAPD increase and small QT prolongation of about 10% (*Chapter 5*). In two additional dogs we measured activation recovery intervals at about 50 sites of the LV and RV endocardium, both before and after the remodeling period. We had hypothesized that within the LV the later-activated sites would display longer repolarization duration, but this could not be demonstrated; activation recovery intervals became inversely related to activation time, with shortest repolarization in the latest activated region. CRT was not applied in this study.

Study limitations

We cannot exclude that arrhythmogenic outcome might (further) change if the duration of DVA is prolonged. We studied DVA for a relatively short period of four weeks, to focus on arrhythmia susceptibility, which has been reported to be present as early as two weeks after creation of AV-block in dogs, whereas structural remodeling, also in isolated LBBB, requires more than eight weeks to fully develop.[8,9,18,40] DVA was created in different ways. In dogs, but also in humans, the DVA pattern during RV apical pacing in normal hearts is known to be quite similar to the DVA pattern during isolated LBBB.[9,10,41] The sample size of subgroups was too small to evaluate whether differences in arrhythmogenic outcome are present between LBBB and RV pacing.

The used screw-in LV epicardial pacing lead, may have resulted in somewhat more physiological LV activation and therefore possibly less (acute) proarrhythmic effect than true epicardial LV stimulation, as mostly used in patients. But purpose of this part of the study was to obtain adequate resynchronization, similar to previous studies performed in Maastricht.[8]

$T_{peak\text{-}end}$ interval during pacing and especially biventricular stimulation need to be interpreted with caution as this may not adequately represent dispersion of repolarization.

Due to interfering P waves during AV-block and lack of software to semi-automatically measure QT interval, we were not able to measure T25 from the QT-interval. The latter may be more attractive for clinical measurements.

Conclusion

Chronic, isolated DVA without bradycardia increases the susceptibility to dofetilide-induced arrhythmias and this is reversible after chronic CRT. Electrophysiological surrogate parameters reflecting repolarization duration and variability at baseline were unable to detect the proarrhythmic remodeling, but the change in time required to obtain a 25 ms increase of MAPD (T25) implies that electrical remodeling contributes to the increased susceptibility, while it remains uncertain whether this is reversed by CRT.

Clinical implications

This study suggests that DVA should not only be prevented to preserve pump function, but also to prevent proarrhythmic electrical remodeling. This proarrhythmic remodeling occurred within four weeks and was not reflected in changes in electrophysiological parameters, like QT and JT, and therefore these parameters might underestimate the risk of TdP / triggered ventricular arrhythmias in patients with DVA, but T25 was valuable for detection of electrical remodeling. Finally, this study suggests that CRT is beneficial to reverse the proarrhythmic effect of isolated DVA, but important limitation is that CRT is currently mostly applied in the presence of comorbidity and after heart failure has developed; therefore, more research is required before CRT can be heralded as anti-arrhythmic against triggered arrhythmias in patients.

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SUPPLEMENTAL METHODS

Anesthesia, perioperative care and electrophysiological recordings

After overnight fasting, about thirty minutes after premedication (methadone 0.4 mg/kg, acepromazine 0.4 mg/kg and atropine 0.5 mg i.m.), anesthesia was induced with pentobarbital sodium (25 mg/kg i.v.), followed by endotracheal intubation and mechanical ventilation. Respiratory rate was set to 12-14/min and end-tidal carbon dioxide concentration was continuously monitored to set tidal volume. Anesthesia was maintained by isoflurane 1.5%, in a 1:2 mixture of O_2 and N_2O. A thermal mattress (39°C) was used to reduce perioperative hypothermia and saline (500 mL NaCl 0.9% i.v.) was administered to prevent volume depletion. Preoperatively dogs received ampicillin 1000 mg i.v. and, if a thoracotomy was performed, also meloxicam 0.2 mg/kg s.c. Postoperatively, ampicillin 1000 mg i.m. and the analgesic buprenorphine 0.3 mg i.m. were administered. Because of suspected hypotension during induction in some experiments, if possible, blood pressure was measured continuously via the artery of the left or right ear. If severe hypotension occurred, this was treated with additional saline infusion (up to 500 ml i.v.) and temporary adrenaline infusion if necessary (1 mg dissolved in 100 ml saline, i.v.). Defibrillator patches were attached in advance and connected to a defibrillator.

A standard 6-lead ECG with four (or three during thoracotomy) precordial leads was recorded in all experiments and the dogs were positioned in left lateral recumbent position during all these recordings under anesthesia. MAP measurements were performed at least 20 s after (re)positioning and catheters were not repositioned after start of dofetilide. If the paced cycle length was changed, measurements were performed at least 3 minutes later.

Implantation of pacing leads and devices

In 15 dogs an (attempt for) implantation of a high-septal lead was performed in a preparatory operation in which also the other transvenous leads and the device were implanted. A transvenous approach via the jugular vein was used (SelectSite C304-S59 deflectable catheter system, Medtronic Inc.) with the associated pacing lead (SelectSecure 3830, Medtronic). If successful, these dogs were included for the study, in groups 1 and 2 (*Figure 1B*). Primary aim was long-term direct His-bundle pacing combined with AV-block by proximal His-bundle radiofrequency ablation, but this was established in only one dog. High-septal pacing was also accepted, provided that evidence of DVA was present: QRS width at least 100 ms after LBBB ablation and MAP signals displaying an about 40 ms later LV than RV activation time. Although initial successful HSP pacing was obtained in ten dogs (in one dog after a second implantation using a different implantation method[42]), due to various causes long-term success, required for inclusion for the main study protocol, was only obtained in thee dogs.

After implantation of the high septal lead, a bipolar, steroid-eluting, screw-in, transvenous lead (5568, Medtronic) was implanted in the right atrium and a bipolar, steroid-eluting, screw-in, transvenous lead (5076, Medtronic) was implanted into the RV free wall at the apex. After

checking pacing and sensing parameters of the leads, the leads were tunneled from the neck to the thorax, where the pocket was created for the device (capable of DDD pacing; Vitatron, The Netherlands; different models). Leads were extended if necessary (6984M, Medtronic).

A thoracotomy through the fourth or fifth right intercostal space was performed to implant the CRT system (LV and atrial lead): after thoracotomy and opening of the pericardium, a screw-in, sutureless, unipolar pacing lead (5071, Medtronic) was implanted in the epicardial LV lateral wall, preferably at 1/3 from the base to the apex, but the lead was repositioned if phrenic nerve stimulation was present at high output or pacing parameters were not acceptable. A steroid eluting, bipolar pacing epicardial pacing lead (4968, Medtronic) was sutured on the right atrium and this lead, the LV electrode and the RV apical electrode, that had been implanted transvenously in the previous experiment, were then connected to a CRT device (Consulta CRT-P, Medtronic). Additionally, as a backup, in some dogs a second, custom-made electrode with 10 mm screw-in electrode, that was isolated, except for the most distal tip (BRC, Maastricht, Netherlands), was screwed into the RV apex from epicardium to (sub)endocardium, and the connector protected with a plug and sutured close to the pocket at the right side of the thorax. During the follow-up of the study this lead was used in one dog, while re-implantation of a transvenous pacemaker lead was performed in another dog that was used in the serial analyses.

In all three groups the right or left femoral artery and vein were dissected and the MAP catheters (Hugo Sachs Elektronik, Germany) were inserted via sheaths to record the normally conducted sinus rhythm.

In groups 1 and 2, next LBBB ablation was performed. The right carotid artery was dissected and a sheath was introduced. Sheaths were flushed with heparin in saline (5000 I.U./500 ml). An ablation catheter (MarinR, Medtronic) was introduced via the carotid artery in the LV. Then the left bundle-branch deflections were anatomically mapped with fluoroscopy and radiofrequency ablation (30 W for 20 s) was performed, starting at the most proximal site. Ablation was aborted if the temperature did not reach at least 43°C, PQ prolonged, ectopic beats occurred for more than five seconds, or if LBBB was not induced within five to ten seconds. Diagnosis of LBBB was based on ECG morphology (QRS widening to about 100 ms and positive in lead II).

In group 2 and 3, complete AV-block was created after the LBBB ablation and after closing the thorax, respectively. The RV MAP catheter was removed and via the femoral vein, an ablation catheter (MarinR, Medtronic) was positioned in the atrial area and the His-deflection was anatomically mapped; requirement was an atrial:ventricular amplitude >2:1. Especially in group 2 care was taken to ablate as close to the AV-node as possible, based on the mapping. Ablation was performed at 25 W for 30 s.

In groups 2 and 3, DVA was initiated immediately after AV-ablation by pacing in VDD mode (or DDD mode with the lower tracking rate at 30 bpm), with the high-septal and RV apical lead in group 2 and 3 respectively and a sensed AV-delay of usually 100 ms, aimed to obtain a similar PQ as during normally conducted sinus rhythm before ablation. In all three groups ECG recording and LV and RV MAP recording were performed at acute DVA.

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Chapter 9

General discussion

Thom R.G. Stams

9

This thesis focused on the risk prediction of Torsade de Pointes (TdP), the role of ventricular activation for arrhythmogenesis and the influence of ventricular activation on risk prediction. First the role of ventricular activation for arrhythmogenesis will be discussed, then the risk prediction.

Quantification of TdP arrhythmogenesis

In the dog with chronic, complete AV-block (CAVB), primary outcome is often the TdP incidence, a dichotomous parameter with cut-off value of ≥3 TdP episodes in 10 min. This parameter can be cumbersome, because it is only useful to evaluate relatively large effect sizes (due to the typically small sample sizes). Several additional parameters that describe severity have been used, including the number of ectopic beats, runs of ectopic beats and cardioversions; the average or cumulative duration of TdP episodes and TdP induced during programmed electrical stimulation.[1-6] To refine the quantification of spontaneously induced arrhythmias, we introduced arrhythmia score in *Chapter 6*. This parameter combines many aspects of severity into a single score: the induction of ectopic beat and short runs of ectopic beats, episodes of TdP and their duration and electrical cardioversions. Because it provided more detail than TdP incidence, arrhythmia has been used to quantify arrhythmic outcome in the subsequent studies.

Using arrhythmia score, in *Chapter 6* we showed that also at acute AV-block dofetilide caused a small but significant increase of arrhythmogenesis, when serially compared with baseline (from 1.0 [1.0-1.3] to 1.8 [1.0-3.6] (median [interquartile range]); P=0.042; n=10).

In *Chapter 8*, again using serial analyses we showed that chronic, isolated dyssynchronous ventricular activation (DVA) caused an increase of arrhythmia score (from 1.3 [1.3-1.3] to 3.3 [2.0-37]; P=0.021, whereas cardiac resynchronization therapy (CRT) in dogs with chronic DVA could decrease the score from 26 [4.3-39] to 1.8 [1.5-3.6]; P=0.022.

In *Chapter 2* we experienced difficulties when evaluating the proarrhythmic effect of K201. If we would apply arrhythmia score the increase would be significant for the higher dose: from 1.3 [1.0-1.5] at baseline to 3.3 [1.3-4.2]; P=0.02; whereas no difference was present for the lower dose: from 1.3 [1.0-1.5] to 1.0 [1.0-2.2], P=NS (statistics: two-way repeated measures ANOVA with post-hoc Bonferroni corrected all pairwise comparisons and logarithmic transformation of arrhythmia score). This further strengthens the impression that the drug (at the higher dose) was proarrhythmic, although the proarrhythmic potential of K201 was much lower than that of dofetilide.

Arrhythmogenesis of TdP: importance of ventricular activation pattern during remodeling

In the canine CAVB model the adaptations of the cardiac ventricles that are associated with the restoration of cardiac output, the so called ventricular remodeling, can be roughly separated into three distinct remodeling processes: structural remodeling (biventricular hypertrophy), contractile remodeling and electrical remodeling. Especially electrical remodeling has been linked to the enhanced TdP susceptibility. In general remodeling that is associated with an increased risk of

TdP is associated with QT prolongation, for example also in chronic heart failure. The CAVB model is a model of 'acquired long QT syndrome' (see also the overview of the model in the preface and a detailed review by Oros, et al.[6]).

The bradycardia-induced volume overload in AV-block dogs was considered more important contributor to proarrhythmic remodeling than the abnormal ventricular activation pattern and AV-dyssynchrony.[7,8] In 2007, Winckels et al. showed that the idioventricular rhythm contributes to proarrhythmic remodeling.[2] Immediately after creation of AV-block, high-septal pacing (HSP) at lowest captured rate was initiated with the aim to maintain a physiological activation pattern. This significantly reduced the severity of TdP episodes, compared to a control group of unpaced AV-block dogs with chronic idioventricular rhythm. Important limitation is the absence of detailed information about the differences in ventricular activation pattern. As a consequence it remains unclear whether the effect of HSP was linked to reduction of dyssynchrony of the left ventricular (LV) activation pattern. In their study, measurements under general anesthesia showed a prolonged QRS in both groups (QRS of 88-96 ms during idioventricular rhythm versus 80 ms during HSP). In addition, during HSP the initial part of activation, from pacing spike to 'QRS onset', was excluded for QRS measurements. The uncontrolled focus in idioventricular rhythm dogs represents another limitation in the study: the comparison was limited to single measurements under general anesthesia, while in dogs with idioventricular rhythm the focus might have changed over time during the remodeling period. Thus, it was shown successfully that altered activation influences ventricular remodeling during chronic bradycardia, but the altered ventricular activation pattern due to QRS widening in HSP dogs may have biased the ability to evaluate the isolated effect of bradycardia. In this thesis we limited the studies to the role of isolated dyssynchronous ventricular activation, its reversibility with CRT (*Chapter 8*) and the role of DVA combined with bradycardia (*Chapter 5*). Therefore, further research would be required to investigate the proarrhythmic effect of chronic, isolated bradycardia in absence of abnormal ventricular activation.

Winckels et al. suggested that the predominant LV activation in idioventricular rhythm dogs enhances the LV electrical remodeling.[2] This hypothesis was tested in *Chapter 5*: bradycardia and DVA were combined, with the aim to cause a late LV activation to reduce LV electrophysiological remodeling and TdP arrhythmogenesis. Because arrhythmogenesis of TdP was not reduced, a control group of dogs with left sided activation was not added. Instead, in two dogs the intraventricular heterogeneity in repolarization was investigated, while the proarrhythmic effect of chronic, isolated DVA was investigated in another study (*Chapter 8*). This revealed that DVA results in proarrhythmic remodeling independent of the presence of chronic bradycardia, while bradycardia worsens DVA induced proarrhythmic remodeling.

Arrhythmogenesis of TdP: effect of acute changes in ventricular activation

Normal ventricular activation is initiated in the sinus node and conduced to the ventricles via the specialized ventricular conduction system. This results in a rapid and quite synchronous ventricular activation. In addition AV-synchrony is present, and the physiological AV-delay allows a

timed atrial contribution to ventricular filling.

Bradycardia is a well-known acute risk factor of TdP, while high-rate pacing can prevent TdP and is used clinically as a treatment of drug-induced TdP. The anti-arrhythmic effect of high-rate pacing has also been shown in the CAVB model.[9] On the other hand, sudden rate accelerations by pacing can induce TdP.[1] Also short-long short sequences are a risk factor for drug-induced TdP induction; the same underlying mechanism has been suggested for the relation between ventricular extrasystoles and TdP.[10]

Acute changes in activation pattern may also be proarrhythmic, but this has only partly been addressed in this thesis. In *Chapter 8*, dofetilide was administered acutely after creation of AV-block/DVA and surprisingly caused TdP in two out of eleven dogs, even lethal in one dog. However, no difference in arrhythmia score was observed compared with the acute AV-block dogs in *Chapter 6* and also after pooling all data no correlation between QRS width and arrhythmic outcome was observed (data not shown). Therefore, in *Table 1*, which provides an overview of the differently proarrhythmic remodeled dogs, these groups were pooled.

Especially LV epicardial pacing in CRT has been associated with an acute increased risk of TdP.[11,12] We have tried whether temporary CRT or LV stimulation (each for 1 min) 15 min after dofetilide administration in dogs with proarrhythmic remodeling due to chronic DVA could acutely induce TdP, but TdP was never induced (unpublished data).

The effect of AV-synchrony on arrhythmogenesis was studied in *Chapter 7*, although this study was primarily aimed at beat-to-beat variability of repolarization to predict TdP (see below under '*Risk prediction of TdP*'), no difference in arrhythmogenic outcome after dofetilide was observed, between experiments during constant PQ (lower preload variability) and alternating PQ (higher preload variability).

Risk prediction of TdP

Most often used risk predictor for TdP is repolarization prolongation, clinically most commonly evaluated by heart-rate corrected QT interval (QTc). Clinically QT is an independent risk factor of sudden cardiac death in patients without DVA.[13,14] However, QT interval is a cumbersome parameter: the measurement is troublesome[15] and the amount of QT prolongation does not always correlate with proarrhythmic risk; QT prolongation can be even accompanied by reduction of TdP risk and a short-coupled variant of TdP exists where TdP occurs without QT prolongation.[16-18] Therefore additional tests and parameters have been introduced to improve the quantification, including parameters that quantify spatial dispersion (like the interventricular difference in monophasic action potential duration (ΔMAPD) and the interval from peak to end of the T wave ($T_{peak\text{-}end}$).[19,20] More recently beat-to-beat variability of repolarization duration has been introduced as risk predictor in the CAVB dog model, where it showed additional value over QT (and ΔMAPD).[6,21] The predictive value of beat-to-beat variability of repolarization for ventricular arrhythmias has also been shown in a variety of clinical cardiac populations.[22-29]

We showed that underlying mechanism of the increased short-term variability of LV MAPD

Table 1. Overview of dogs with differences in remodeling, related to differences in ventricular activation and bradycardia

	aAVB	DVA #2 + CRT	HSP	DVA #2	DVA	IVR
Remodeling period						
DVA	-	+ / -	- (?)	+	+	+ (?)
Bradycardia	-	-	+	-	+	++
AV-dyssynchrony	-	-	+	-	+	+
Arrhythmogenesis						
TdP, %	8	13	30	29	75	74
AS mean	4.4	4.5	14	14	28	41
median	1.3	1.8	1.3	3.3	23	47
[IQR]	[1.3 - 2.3]	[1.5 - 3.6]	[1.0 - 24]	[1.5 - 29]	[8.2 - 41]	[10 - 65]
n	24	8	10	14	8	19
EP, baseline						
QRS, ms	105 ± 21	96 ± 10	123 ± 12	117 ± 12	118 ± 12	94 ± 15
LV MAPD, ms	240 ± 22	246 ± 15	269 ± 16	252 ± 26	255 ± 23	299 ± 44
STV_{LV} median, ms	0.8	0.4	1.0	0.6	0.5	1.4
[IQR], ms	[0.4 - 1.0]	[0.3 - 0.5]	[0.9 - 1.7]	[0.3 - 0.7]	[0.5 - 0.8]	[0.9 - 1.6]
EP, dofetilide						
LV MAPD, ms	335 ± 72	376 ± 35	354 ± 28	344 ± 53	411 ± 70	439 ± 88
STV_{LV} median, ms	1.4	1.4	2.0	1.6	2.4	2.1
[IQR], ms	[0.8 - 1.6]	[1.1 - 1.4]	[1.7 - 2.3]	[1.3 - 2.0]	[1.6 - 4.8]	[1.4 - 3.5]
T25, min	2.1 ± 0.4	2.1 ± 0.5	1.9 ± 0.4	1.8 ± 0.3	1.5 ± 0.3	1.4 ± 0.4
Thesis Chapter	6, 8	8	6	8	5, 6	6

The measurements were obtained from the experiments under general anesthesia, during bradycardia (VVI pacing at a constant ventricular rate of 60/min or idioventricular rhythm), before (baseline) and after administration of dofetilide (0.025 mg/kg/5min). Data are sorted based on severity of arrhythmogenesis after dofetilide administration.

Abbreviations: aAVB, acute AV-block, pooled data of the unpaced/paced dogs from *Chapter 6* and the RVA paced dogs from *Chapter 8*. AS, arrhythmia score; CRT, cardiac resynchronization therapy (performed for eight weeks, after four weeks of DVA without bradycardia); DVA #2, dyssynchronous ventricular activation, second study (*Chapter 8*) in which DVA without chronic bradycardia was studied; EP, electrophysiological parameters; HSP, high-septal pacing; IQR, interquartile range; IVR, idioventricular rhythm; LV MAPD, left ventricular monophasic action potential duration at 80% repolarization; STV_{LV}, short-term variability of LV MAPD; T25, time required to obtain 25ms-increase after dofetilide; TdP, Torsade de Pointes.

(STV) after proarrhythmic remodeling and dofetilide is the variation of preload: only during increased preload variation the STV increased. The preload variation was induced by alternating PQ interval and to a lesser extent also by mechanical ventilation (*Chapter 7*).

Influence of ventricular activation pattern on electrophysiological parameters used for risk prediction

In the literature some conflicting data exist concerning the isolated effect of changes in ventricular activation pattern: in some studies electrophysiological adaptations were enhanced in the earliest activated regions, compared to latest activated regions,[30,31] whereas in other studies most severe remodeling was shown in the latest activated regions.[32,33] Increasing evidence becomes available that suggests that mechano-electrical feedback in the late activated region (due to stretch as a consequence of the dyssynchronous electrical activation) may be responsible for the adaptations responsible for the adaptation processes (this is often called 'cardiac memory' because the adaptations are linked to transient T wave changes after restoration of activation, that slowly subside).[33-35]

Our data are most in agreement with the first groups, because we observed no signs of repolarization prolongation in the latest activated regions: (1) this would have been discernible in the QT interval measurements, unless the area was too small to allow electrical detection (end of the T wave); (2) in the repolarization mapping study (*Chapter 5*; n=2) we observed a clear negative correlation between activation time and repolarization duration (evaluated with activation recovery intervals), independent of transmural site.

These data suggested that in the AV-block dog the changes in action potential duration are primarily caused by the activation pattern; therefore the same masking issue might have been present in the dogs with chronic isolated DVA (*Chapter 8*): If in these dogs the earliest activated LV regions would prolong, like we had observed in the dogs with chronic bradycardic RVA pacing, the changes in repolarization could have been masked by the later activated regions when evaluating QT/JT interval. To test this hypothesis, we evaluated the effect of temporary biventricular paced activation (CRT), before and after remodeling due to DVA, which had been performed serially in eight dogs. The biventricular stimulation results in relatively later activation of the early activated LV region, because the LV activation was initiated simultaneously with the RV activation from the later activated LV region of the heart. The results confirmed the hypothesis: acutely after initiation of DVA, JT intervals were not prolonged during CRT (255±20 ms) compared to DVA (251±19 ms), whereas after four weeks remodeling, JT intervals were significantly prolonged by CRT (298±23 ms, versus DVA: 259±28 ms; $P<0.001$; *Figure 1*).

STV at baseline was not closely related to arrhythmogenic outcome: especially chronic DVA was not associated with an increased STV (*Table 1*). Moreover, no reference values are available for AV-block dogs: in other studies, considerable variation in baseline values is present.[36] This may be related to differences in methods of anesthesia, recording or analysis, but we still failed to find the reason.

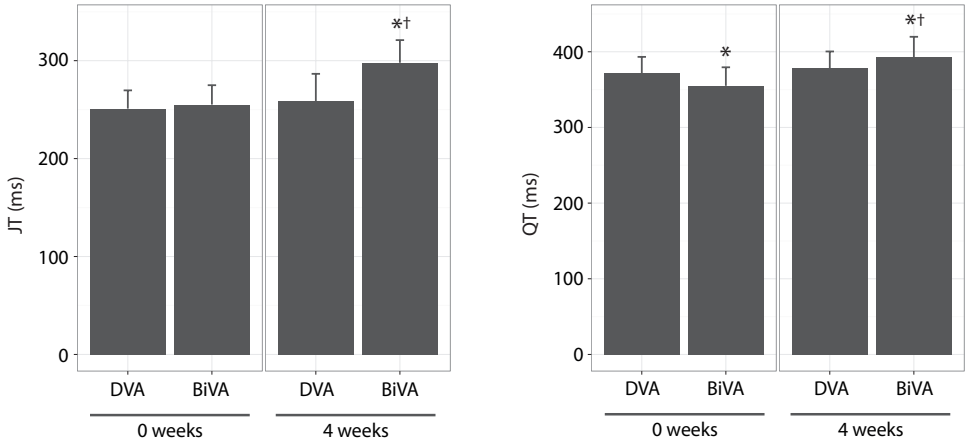

Figure 1.

Effect of a temporary biventricular paced activation (BiVA) on JT and QT intervals acutely after initiation of dyssynchronous ventricular activation (DVA) and AV-block, and after four weeks remodeling due to isolated DVA without bradycardia. The tests were performed under general anesthesia during a constant ventricular rate of 60/min.

*, P<0.05 vs. DVA; †, P<0.05 vs. 0 weeks.

Building on the findings in *Chapter 7*, we evaluated whether pacing with alternating PQ interval could increase STV while maintaining DVA, by RVA pacing at a rate of 60/min: STV was 0.7 [0.4-1.7] at acute DVA and 0.5 [0.4-1.1] at chronic DVA; P=NS; n=7. A possible reason could be that not only preload variation is required, but also the right spot within the ventricle. (In *Chapter 5*, in dogs which bradycardia and chronic DVA, the mapping revealed quite significant heterogeneity of STV, but also highest values in the early activated areas). The longer repolarization duration in early-activated regions may increase the predictive value of STV, for example related to the 'negative electromechanical window' (Chapter 4), which means that repolarization ends after the end of mechanical relaxation: this may allow more mechanical influence on repolarization (mechano-electrical feedback; *Chapter 7*).

After dofetilide, the association between STV and arrhythmogenic outcome seemed to improve (*Table 1*). However, the initial increase of LV MAPD seemed to be the best predictor, also because this parameter was determined earlier in time. The increasing LV MAPD after dofetilide may by itself contribute to STV. Therefore, a correction was applied; this was also the original reason to measure the speed of increase of LV MAPD during dofetilide. In *Figure 2*, this corrected STV ('$STV_{LV,C}$') is presented for the data from *Chapter 6*. The exponential fit, which was used for calculation of T25 was subtracted from the original data to remove the gradual increase of MAPD. Then '$STV_{LV,C}$' was calculated, similarly to regular STV measurements (based on the absolute beat-to-beat differences). The figure confirmed that T25 is an earlier predictor of TdP than $STV_{LV,C}$ after dofetilide: although only in the IVR and RVA dogs an increase of $STV_{LV,C}$ seemed to be present, after 2 min RVA dogs still showed the lowest values.

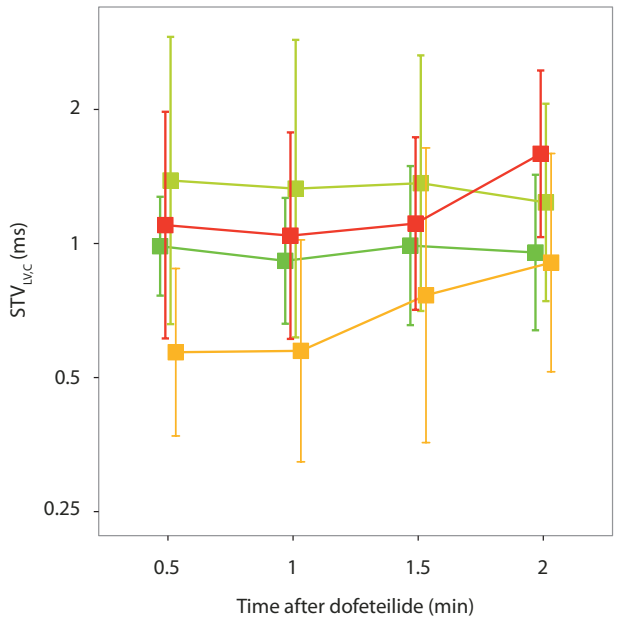

Figure 2

Time-dependent increase of short-term variability of left ventricular (LV) monophasic action potential duration at 80% repolarization (MAPD), during the first 2 minutes after start of dofetilide infusion (the time window used for T25 calculation).

A correction for the exponentially increasing MAPD was applied by subtracting the exponential fit of LV MAPD data from the original data ('$STV_{LV,C}$'). Presented data are from the groups in *Chapter 6*: idioventricular rhythm (red); chronic bradycardic right ventricular apical pacing (orange); chronic bradycardic high-septal pacing (light green); and unremodeled acute AV-block dogs (dark green). Colors correspond to arrhythmia severity (see *Chapter 6*). Note the logarithmic y-axis.

Induction of TdP: relation with repolarization duration

In *Chapter 5* we investigated the relation between spatial dispersion within the LV and the induction of ectopic beats (*Chapter 5: Figure 5*). If we take a closer look, the induction of the ectopic beats was associated with acute shortening of the long activation recovery interval, abolishing the spatial dispersion of repolarization moments in that area. This was not only rate-dependent, because the spatial dispersion reappeared during the third beat. Speculating further on this single observation, this might be relevant as it may explain the beat-to-beat change of focus of TdP, provided that the spatial dispersion is involved in the mechanism of induction of focal activity and also during the subsequent beats.

Also interesting is that in the dog a close correlation between the ARI at baseline and after dofetilide was observed. Because the slope was larger than 1 (1.6), the pre-existent heterogeneity was amplified. These findings do not necessarily implicate that precise baseline measurements of action potential duration and heterogeneity would suffice to determine arrhythmia vulnerability. Especially during chronic DVA, we observed a relatively short LV MAPD, but also a shortened T25, thus indicating that the (initial) increase in LV MAPD after dofetilide was relatively high in spite of the short baseline LV MAPD (*Table 1*). Also in other studies differences in repolarization prolongation after I_{Kr}-block have been described (e.g. by Kaab et al. and Kozhevnikov et al.).[37,38] This may implicate that the slope of the correlation between action potential duration at baseline and after dofetilide, as a measure of amplification of heterogeneity, is variable (e.g. depending

on the severity of pre-existent remodeling or other interindividual differences). Thus, besides the spatial dispersion of repolarization at baseline, this may be important for proarrhythmic risk prediction upon I_{Kr} block.

Other factors could be relevant for arrhythmogenesis as well, including disturbances in calcium handling and in myocardial structure. To evaluate their relevance, further research is required.

Clinical implications

Our data suggest that DVA, with or without chronic bradycardia, should not only be prevented to preserve pump function, but also to prevent proarrhythmic electrical remodeling. We studied continuous DVA activation, induced by continuous RV apical pacing or LBBB, but DVA may also occur in other circumstances, for example in the setting of ventricular extra systoles.[39] Further research is required to determine the relevance of the cumulative duration, time course and severity of DVA and of the presence of comorbidity.

The more preferential prolongation of repolarization in early activated LV regions, suggests that precautious use of baseline parameters is required. Also STV was not increased by chronic DVA with or without bradycardia, even in the setting of alternating PQ (alternating preload). Reversal of the activation sequence may be a feasible method to detect proarrhythmic remodeling in patients with DVA. Measurement of T25 during I_{Kr}-block may be considered to more reliably quantify the arrhythmic risk, for example in patients with suspected arrhythmic syncope or before initiation of class III drugs.

Our data also suggested that the observed QT prolongation and acute proarrhythmia after onset of CRT[11,12] may be caused by the underlying chronic DVA that is typically present in these patients. In *Chapter 8*, we showed that CRT applied for eight weeks had largely reversed DVA-induced proarrhythmia. In *Chapter 5* we showed that the spatial dispersion seemed to be important for induction of TdP after chronic DVA. To prevent this issue upon initiation of CRT in patients with a history of DVA, maybe a slowly decreasing VV-delay (from RV<LV to the usually required LV=RV or RV>LV) or a temporary higher heart rate could be beneficial during the acute phase of CRT.

In *Chapter 7*, we described that applying controlled preload variation may be a safe way to increase the predictive value of STV for ventricular arrhythmias.

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ENGLISH SUMMARY

Role of electrical activation of the heart

The heart is normally paced by the sinus node, in humans at a rate of about 60-100 beats per minute. The sinus node is located in the right atrium. Conduction of this electrical 'signal' over the tissue is required for contraction, a process called excitation-contraction coupling. The atria and ventricles are electrically isolated, except for a small area, which is slowly conducting: the AV-node. Therefore, a delay occurs before the ventricles are activated via the specialized His-Purkinje system. This well-timed delay allows an atrial contraction that improves ventricular filling before the contraction (preload). The ventricles are most important, because they pump the blood to the body (left ventricle) or lungs (right ventricle). A good pump function requires a well-coordinated ventricular contraction and because of the excitation-contraction coupling, also well-coordinated electrical activation. Both asystole (absence of electrical activations) and fast ventricular arrhythmias can acutely interrupt the pump function and thereby cause loss of consciousness in seconds and sudden cardiac death in minutes.

If the conduction through the left bundle branch of the ventricular conduction system is blocked (disease) or if the heart is paced from the right ventricle (the most commonly applied method of ventricular pacemaker therapy), the contraction of the left ventricle becomes dyssynchronous, resulting in less efficient pump function. On the longer term this is associated with development of heart failure, in a minority of patients. Symptomatic, chronic heart failure is a severe disease with a mortality of about 50% within 5 years after onset. Not only terminal pump failure itself is an important cause of death, but also sudden death due to ventricular arrhythmias, which can also occur when pump function is not severely decreased yet.

Torsade de Pointes

Torsade de Pointes (TdP) is an example of a fast ventricular arrhythmia that can result in sudden death. TdP most commonly ends within seconds but typically several episodes occur and episodes can have a long duration or degenerate into ventricular fibrillation. Ventricular fibrillation will result in sudden cardiac death if defibrillation is not applied immediately. To diagnose TdP, an electrocardiogram (ECG) recording is required to record the electrical activity of the heart; TdP has a typical sinusoid pattern.

The ECG is also very important for risk prediction of TdP. An ECG shows the atrial and ventricular electrical excitation (P wave and QRS complex, respectively) and also the ventricular repolarization (T wave) [*Figure 1*]. The total duration of ventricular electrical activity (from QRS onset to end of T wave: QT interval), is used as a measure of ventricular repolarization, because the repolarization is a much slower process than the excitation. Typically TdP occurs in the setting of a prolonged QT interval, due to a delayed repolarization process. QT prolongation can be caused by congenital factors, most importantly the congenital long QT syndrome, caused by a genetic mutation of one of the involved ion channels, but also caused by acquired factors, e.g.

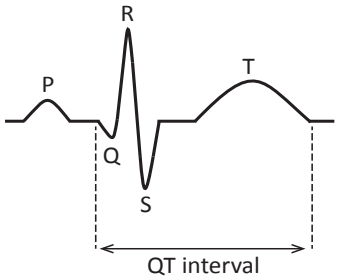


Figure 1.

Schematic representation of the normal electrocardiogram (ECG)

(From Varkevisser, 2014, Thesis: *Is if safe? Adverse drug effects and cardiac arrhythmias*, with permission)

chronic heart failure and drugs. Drugs that block one or more of the ion currents that underlie normal ventricular repolarization are often involved in the induction of the arrhythmia. The block of repolarizing currents can be an intended effect, in anti-arrhythmic drugs like dofetilide and sotalol, but is commonly an adverse effect of the drug (secondary pharmacology). Usually only in a very low fraction of patients TdP will be induced by the drug, because a significant risk of life-threatening arrhythmias is usually not acceptable.

Repolarization prolongation is not only used for risk prediction of TdP in patients, but is also important in safety pharmacology to evaluate the proarrhythmic risk of drugs. Because TdP itself is usually a rare adverse event, the arrhythmia is often not detected in healthy animals or humans and therefore a central question in the safety evaluation is: does the drug prolong repolarization? Unfortunately, repolarization prolongation is not closely associated with TdP risk and some drugs that prolong QT are even safe (for example the antibiotic moxifloxacin).

One way to improve safety evaluation, especially if concerns about safety exist due to QT prolongation, is to investigate the proarrhythmic effect in animal models that are more sensitive to TdP arrhythmias. Important model is the chronic, complete AV-block dog model. In these dogs, under general anesthesia, complete AV-block is artificially created. The result is that electrical excitations from the atria are no longer conducted to the ventricles. Instantaneous death due to asystole is prevented due to spontaneously emerging escape rhythm in the ventricles: a physiological backup pacemaker. This spontaneous rhythm (idioventricular rhythm) is much slower (bradycardia) and as a consequence, the amount of blood that is pumped out by the heart per minute (the cardiac output) is reduced. To compensate, the heart adapts by contracting more powerful (increased contractility) and by becoming larger and increasing the amount of muscle fibers in the tissue (hypertrophy). This 'remodeling' of the heart results in almost complete restoration of the cardiac output, but is associated with electrical remodeling. This is visible as QT prolongation on the ECG and is associated with an increased susceptibility to TdP. The model is used in research to evaluate the safety of novel drugs and to study the underlying mechanisms of TdP. Drugs that were free of TdP even if prolonging QT, did not result in TdP in the model, while drugs that cause TdP in (a minority of) humans, resulted in TdP in up to 75% of these dogs. The drug dofetilide is used as a reference compound (positive control) in the model, because this drug results in the highest inducibility of 75%, within 15 minutes after start of infusion under general anesthesia. Dofetilide specifically blocks one potassium ion current that is important for

ventricular repolarization. This drug is used in the USA to treat patients with atrial fibrillation, an atrial arrhythmia that is very common, especially at older age. Due to the significant risk of TdP in patients treated with dofetilide, strict monitoring of the patients is required during initiation of the therapy. Also other drugs, like sotalol which is also used in the Netherlands, are associated with TdP as side effect. The risk of TdP is one of the reasons that novel drugs are being developed for the treatment of atrial fibrillation. One of those drugs is K201. This drug's primary mode of action is prevention of leakage of calcium from the intracellular Ca^{2+} storage compartment (the sarcoplasmic reticulum) during diastole. This calcium is important for contraction (systole) but especially the leakage during diastole may cause arrhythmias. However, K201 also blocks other ion channels, including the potassium ion current that is also blocked by dofetilide. In *Chapter 2*, we investigated whether K201 would be anti-arrhythmic against TdP due to its effect on the sarcoplasmic reticulum; however no anti-arrhythmic effects against TdP were observed, while at the higher dose even some proarrhythmia was observed: one out of seven dogs showed spontaneous TdP episodes after K201 treatment.

Because QT interval is not a reliable parameter to predict risk of TdP, we also did an additional measurement using a monophasic action potential catheter that was inserted via the aorta in the left ventricle and positioned on the wall of the left ventricle. Increased beat-to-beat variability of left ventricular repolarization ('instability of repolarization') was observed after the higher dose of K201. This provided additional evidence that the higher dose was not safe.

In the chronic AV-block dog model, calcium channel blockers are a highly effective treatment against TdP, but drawback is the negative inotropic effect: they usually impair the pump function of the heart, because calcium is required for contraction. This is especially a drawback if this treatment would be applied in patients with heart failure. On the other hand, these patients have an increased risk of life-threatening arrhythmias like TdP. This is discussed in *Chapter 3*, which is a commentary to a study of Milberg et al. who studied the calcium channel blocker verapamil to suppress TdP arrhythmias in rabbits with heart failure.

In *Chapter 4*, the relation between the duration of mechanical contraction and QT interval was studied, because this had been shown to be a better predictor of TdP than QT interval per se. However, we observed that in the chronic AV-block model in dogs no additional value was present over QT interval measurements.

Dyssynchronous ventricular activation

Most important aim of this thesis was to study the role of pathologically altered ventricular activation in the arrhythmogenesis of TdP. It is known that dyssynchronous ventricular activation (DVA) due to right ventricular pacing or left bundle branch block can decrease pump function and result in ventricular remodeling, but it is unknown whether DVA increases susceptibility to TdP arrhythmias. In *Chapter 5*, the effect of DVA in combination with bradycardia on arrhythmogenesis of TdP was studied in eight AV-block dogs. After the remodeling period, TdP could be induced in 75% of the dogs during a standard dofetilide test. However, the parameters that

we used to quantify electrical remodeling did not show evidence of significant electrical remodeling. For example the duration and beat-to-beat variability of the left ventricular monophasic action potential duration were not increased (compared to acutely after initiation of DVA and bradycardia). Next, we studied whether regional differences within the left ventricle were present. During DVA (in this study by right ventricular apical pacing) the heart is electrically activated from the right ventricle to the left ventricle. This results in an earliest left ventricular activation at the area between left and right ventricle (the septum), whereas the free wall at the base of the heart generally activated latest. Interestingly we found that the remodeling was associated with development of a clear relation between the monophasic action potential duration and the activation time in these two dogs. We also studied the effect of dofetilide administration in one dog and these results suggested that the regional differences in repolarization within the left ventricle were important for arrhythmogenesis of TdP.

In *Chapter 6* the effects of DVA in combination with bradycardia were compared with other groups of dogs from previously conducted experiments: unremodeled dogs (acute AV-block), normal AV-block dogs with chronic idioventricular rhythm and dogs with remodeling due to chronic bradycardia in combination with a more physiological activation pattern (high-septal paced dogs).

In studies in chronic AV-block dogs, usually arrhythmias are quantified as TdP inducibility. This parameter is dichotomous (the outcome is either 'yes' or 'no'). In this study also arrhythmia score was introduced, with the aim to obtain more detailed information of arrhythmogenesis. This score can range from 1 to 100 and provided more detailed quantification. In the same study, also the initial prolongation of left ventricular repolarization (monophasic action potential duration) during dofetilide infusion was introduced, to determine whether this was predictive for TdP. This was quantified as time required to obtain a 25ms-increase (T25). The parameter was a good predictor of TdP.

In *Chapter 8*, we studied whether chronic DVA in absence of chronic bradycardia would be proarrhythmic and whether a subsequent period of cardiac resynchronization therapy (CRT) could reverse this. We found that DVA was proarrhythmic, but the outcome was less severe than we had observed during DVA in combination with chronic bradycardia. Analysis of the parameters used for TdP prediction revealed that the proarrhythmic effect of DVA was only linked to a reduction of T25. We also found that CRT was anti-arrhythmic against the DVA-induced proarrhythmia.

In *Chapter 7*, we studied the underlying mechanism of beat-to-beat variability of repolarization. We found that the mechanical variation due to preload variation caused small differences in the repolarization from beat to beat (mechano-electrical feedback), but only after proarrhythmic remodeling due to chronic complete AV-block. This was enhanced after administration of dofetilide. Further we showed that this may be caused via stretch-activated channels in the heart, because streptomycin (a drug that blocks stretch-activated channels) interrupted the mechano-electrical feedback.

The final chapter (*Chapter 9*) is a general discussion of the thesis.

NEDERLANDSE SAMENVATTING

Rol van elektrische activatie in het hart

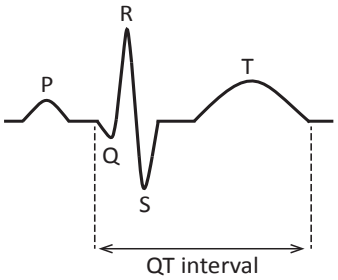
Het hart wordt normaliter elektrisch gestart vanuit de sinusknoop. De sinusknoop bepaalt daarmee het hartritme (bij mensen circa 60-100 per minuut). De sinusknoop bevindt zich in de rechter boezem. Geleiding van het elektrische 'signaal' over de boezems (excitatie) is nodig voor de boezems om te kunnen samentrekken (contractie). Dit proces wordt excitatie-contractie-koppeling genoemd. De boezems en de kamers zijn elektrisch geïsoleerd, met uitzondering van een klein gebiedje genaamd de AV-knoop. De AV-knoop geleidt erg traag, waardoor de kamers pas elektrisch geactiveerd worden nadat de boezems zijn samengeknepen. Omdat de boezems het bloed naar de hartkamers pompen resulteert dit in een toename van de vulling van de kamers voor de contractie (preload). De kamers zijn belangrijker dan de boezems, omdat de kamers het bloed door het lichaam (linker kamer) en de longen (rechter kamer) heen pompen. Zowel asystolie (afwezigheid van elektrische activaties) als snelle aritmieën kunnen acuut de pompfunctie verstoren en daardoor leiden tot bewustzijnsverlies (seconden) en plotse hartdood (minuten).

Het elektrische signaal, afkomstig vanaf de AV-knoop, bereikt de rechter kamer via de rechter bundeltak en de linker kamer via de linker bundeltak. Als de geleiding door de linker bundeltak geblokkeerd is (ziekte) of wanneer een pacemaker wordt geïmplanteerd met een draad in de rechter kamer (de standaard positie voor pacing van de kamer), wordt de linker kamer veel minder synchroon geactiveerd. Dit resulteert in afname van de linker kamer functie en kan heel soms ook leiden tot hartfalen. Hartfalen is een ernstige ziekte: ongeveer de helft van de patiënten overlijdt binnen vijf jaar. Ritmestoornissen zijn een belangrijke doodsoorzaak in patiënten met hartfalen.

Torsade de Pointes

Torsade de Pointes (TdP) is een voorbeeld van een snelle ritmestoornis van de kamers die de pompfunctie acuut verstoort. TdP eindigt vaak spontaan binnen seconden, maar komt vaak meermaals terug en kan ook leiden tot kamerfibrilleren. In dat laatste geval is direct defibrillatie nodig om plotse hartdood te voorkomen.

Het elektrocardiogram (ECG / 'hartfilmpje') is belangrijk om TdP te diagnosticeren. Op een ECG is de elektrische activatie van zowel de boezems als de kamers zichtbaar, als P-golf respectievelijk QRS-complex (*Figuur 1*). Tevens is de repolarisatie van de kamers zichtbaar als de T-golf. De totale duur van de elektrische activiteit van de kamers (vanaf het begin van het QRS complex tot het einde van de T-golf) is het QT interval. Een verlengd QT interval duidt er op dat het risico op TdP verhoogd is. Het QT interval kan verlengd zijn door een aangeboren lang-QT-syndroom, maar ook door bijvoorbeeld ziektes, zoals hartfalen. Ook bestaan er medicijnen die ionkanalen blokkeren die bijdragen aan de repolarisatie van de kamers en daardoor het QT interval kunnen verlengen en in een klein gedeelte van de patiënten TdP kunnen veroorzaken. De blokkade van de ionkanalen kan een bewust onderdeel zijn van de werking, bij medicijnen die gebruikt worden om ritemstoornissen te behandelen (bijvoorbeeld dofetilide en sotalol), maar



Figuur 1.

Schematisch overzicht van het normale electrocardiogram (ECG)

(Bron: Varkevisser, 2014, Proefschrift: *Is if safe? Adverse drug effects and cardiac arrhythmias*, met toestemming)

kan ook een bijwerking zijn.

Het QT interval wordt daarom niet alleen gebruikt om het risico van TdP te bepalen in patiënten, maar ook om de veiligheid van nieuwe medicijnen te onderzoeken: als deze het QT interval verlengen zijn ze mogelijk onveilig. Een nadeel is dat het QT interval geen hele goede voorspeller is van TdP. Sommige medicijnen verlengen namelijk het QT interval, maar zijn toch veilig, zoals het antibioticum moxifloxacine.

Eén van de methoden om te onderzoeken of een nieuw medicijn veilig is, ondanks QT verlenging, is toediening van het medicijn in dieren die heel gevoelig zijn voor de TdP ritmestoornissen. Een belangrijk voorbeeld hiervan is de hond met compleet AV-blok. Onder algehele narcose wordt in deze honden kunstmatig een elektrische blokkade in de AV-knoop gemaakt (AV-blok). Dit resulteert niet in asystolie, doordat de kamers spontaan de functie van de sinusknoop overnemen. Echter, dit ritme is heel traag (bradycardie), waardoor het hart acuut minder bloed per minuut rondpompt (verminderde cardiac output). Hierop gaat het hart zich aanpassen: het gaat krachtiger samentrekken (verhoogde contractiliteit) en wordt groter en dikker (hypertrofie). Dit resulteert in vrijwel volledig herstel van de cardiac output. Echter, dit proces ('ventriculaire remodellering') gaat gepaard met verlenging van het QT interval en resulteert in een sterk verhoogde gevoeligheid voor TdP. Diverse medicijnen zijn onderzocht in dit diermodel. Medicijnen die geen TdP veroorzaken ondanks QT verlenging veroorzaakten ook geen TdP in de honden, terwijl medicijnen met een bekend risico van TdP in patiënten in een groot deel van de honden (tot 75%) TdP veroorzaken. Dofetilide wordt in het model gebruikt als positieve controle, omdat het resulteert in het hoogste percentage TdP: in circa 75% van de honden met chronisch AV-blok kan onder algehele narcose TdP worden opgewekt. Dofetilide wordt in de VS gebruikt voor de behandeling van boezemfibrilleren. Vanwege het risico van TdP is strikte controle van de patiënten nodig bij het starten van de behandeling. Ook in Nederland gebruikte medicijnen, zoals sotalol kunnen soms TdP veroorzaken. Dit is een van de redenen dat er nieuwe medicijnen worden ontwikkeld voor de behandeling van boezemfibrilleren. Een van deze nieuwe medicijnen is K201. Dit medicijn grijpt aan op de calciumvoorraad in de cel (het sarcoplasmatisch reticulum): het kan lekkage van calcium tijdens de rustfase tussen contracties voorkomen. Dergelijke calciumlekkage zou mogelijk een rol kunnen spelen bij het ontstaan van TdP. Anderzijds kan

K201 ook andere ionkanalen blokkeren, waaronder het kaliumkanaal dat door dofetilide wordt geblokkeerd. Dit zou juist proaritmisch kunnen zijn.

In *Hoofdstuk 2* werd het effect van K201 onderzocht in honden met chronisch, compleet AV-blok. K201 bleek TdP niet te kunnen voorkomen en de hogere dosering veroorzaakte zelfs TdP in één van de zeven honden. Omdat het QT interval geen heel goede maat is voor TdP risico, wordt in dit model ook de slag-op-slag variatie van de repolarisatie ('instabiliteit van de repolarisatie') gemeten, middels een katheter die tijdens het experiment tijdelijk in de linker kamer wordt geplaatst (ingebracht via een bloedvat). De slag-op-slag variatie bleek verhoogd te worden door de hogere dosering van K201, wat additioneel bewijs opleverde dat het medicijn onveilig is.

Medicijnen die calciumkanalen blokkeren zijn effectief tegen TdP in de chronisch AV-blok hond. Een belangrijk nadeel is echter, dat calcium nodig is voor de contractie (pompfunctie) en het gebruik van een dergelijk medicament leidt tot verminderde pompfunctie. Anderzijds hebben juist ook patiënten met chronisch hartfalen een verhoogd risico op ritmestoornissen, zoals TdP. Dit wordt besproken in *Hoofdstuk 3*, een commentaarstuk behorend bij een studie van Milberg et al. die de calciumblokker verapamil onderzochten om TdP tegen te gaan in konijnen met hartfalen.

In *Hoofdstuk 4*, werd de relatie tussen de duur van de mechanische contractiefase en het QT interval onderzocht, omdat de combinatie mogelijk een betere voorspeller van TdP zou zijn, dan het QT interval zelf. Echter, dat bleek niet het geval te zijn in het chronisch AV-blok honden model.

Dyssynchrone ventriculaire activatie

Belangrijkste doel in dit proefschrift was het onderzoeken van de rol van abnormale, asynchrone activatie van de linker kamer (dyssynchrone ventriculaire activatie; DVA) voor het ontstaan van een verhoogde gevoeligheid voor TdP. Zowel pacing vanuit de hartpunt van de rechter kamer (rechter kamer apex) als linker bundeltakblok kunnen DVA veroorzaken en de pompfunctie verminderen. In *Hoofdstuk 5*, werd het gecombineerde effect van bradycardie en DVA op het onstaan van TdP ritmestoornissen onderzocht in de AV-blok hond. Na een periode van remodellering, kon TdP worden opgewekt met dofetilide in 75% van de honden. Echter, de parameters die gebruikt worden om het risico van TdP vast te stellen, zoals slag-op-slag variatie van de repolarisatieduur, toonden geen duidelijke aanwijzingen voor elektrische remodellering. In een aanvullend onderzoek werden de regionale verschillen in de linker kamer onderzocht. Er bleek een duidelijke relatie te bestaan tussen het moment van elektrische activatie binnen de linker kamer en het moment van repolarisatie. Een soortgelijk onderzoek werd ook uitgevoerd tijdens toediening van dofetilide om TdP op te wekken. In dit experiment bleken regionale verschillen in repolarisatieduur in de linker kamer een belangrijke rol te spelen bij het ontstaan van TdP.

In *Hoofdstuk 6* werden de data van *Hoofdstuk 5* vergeleken met andere groepen van honden uit eerdere studies, waaronder honden met ongeremodelleerde harten (acuut AV-blok) en reguliere honden met chronisch AV-blok en spontaan (ongepacet) ritme tijdens de remodelleringsperiode. In studies in AV-blok honden is de opwekbaarheid van ritmestoornissen vaak de belangrijkste

uitkomstmaat. Dit is een dichotome uitkomst (ja of nee). In deze studie werd de aritmiescore geïntroduceerd om meer gedetailleerde informatie te verkrijgen over de ernst van de ritmestoornissen. Ook werd de initiële toename van de linker kamer repolarisatie (monofasische actiepotentiaalduur) gemeten tijdens dofetilide infusie, om te bepalen of dit voorspellend was voor TdP. Dit werd gekwantificeerd als de benodigde infusietijd om een 25ms-toename van de repolarisatieduur te verkrijgen (T25). Deze parameter bleek een goede voorspeller te zijn van TdP.

In *Hoofdstuk 8* werd onderzocht of ook chronische DVA zónder bradycardie de gevoeligheid voor dofetilide-geïnduceerde ritmestoornissen kan verhogen en tevens of dit omkeerbaar is middels cardiale resynchronisatietherapie (cardiac resynchonization therapy; CRT). Bij CRT wordt het hart tegelijkertijd gepacet met een elektrode in de rechter kamer en een elektrode in de linker kamer, waardoor het elektrische activatiepatroon synchroner wordt. Chronische DVA bleek de gevoeligheid voor dofetilide-geïnduceerde ritmestoornissen te verhogen, maar minder sterk dan de combinatie van DVA en bradycardie. Het proaritmische effect ging slechts gepaard met een afname van de T25; de andere parameters waren niet significant verschillend. CRT behandeling bleek de verhoogde gevoeligheid voor ritmestoornissen als gevolg van DVA te herstellen.

Zowel in mensen als dieren is slag-op-slag variatie van de repolarisatieduur voorspellend voor ritmestoornissen. Het onderliggende mechanisme van de slag-op-slag variatie is echter niet goed bekend. In *Hoofdstuk 7* werd het onderliggende mechanisme van slag-op-slag variatie van de repolarisatieduur in het AV-blok hondenmodel onderzocht. In deze studie werd aangetoond dat slag-op-slag variatie van preload (dus mechanische variatie) een belangrijke bijdrage levert aan de de slag-op-slag variatie van de repolarisatieduur. Deze bevinding suggereert dat het opleggen van gecontroleerde preloadvariatie de voorspellende waarde voor TdP zou kunnen verhogen, mogelijk ook in patiënten.

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Prof.dr. H.V.M. van Rijen
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Prof.dr. K.G.M. Moons
Prof.dr. F. Prinzen
Prof.dr. J.L.R.M. Smeets

Avram
Atty
Jet
Roel
Peter Oosterhoff
Marcel
Vincent
Jacques
Marti
Bart
Albert
Rosanne

Mathias
Peter Loh
Marieke
Jurren
Geert
Tycho
Fred
Irene
Harry
Arne
Marc
Coert
Jacqueline
Alfonso
Jan Schreuder
Howard Dittrich

Photo: Thom Stams


Alexandre
Bastiaan
Bianca
Hanneke
Helen
Hiroki
Ian
Ivar
Leonie
Lotte
Maaike
Magda
Malin
Marieke
Marien
Marlieke
Martin
Paulien
Rianne
Sanne de Jonge
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Sarah
Shirley
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Teun
Tobias
Tonny
Toon
Yuan
Gerard
Joke
Matthijs
Jeroen
Joey
Jolien
Christian
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LIST OF PUBLICATIONS

Peer-reviewed publications

T.R.G. Stams, V.J.A. Bourgonje, H.D.M. Beekman, M. Schoenmakers, R. van der Nagel, P. Oosterhoff, J.M. van Opstal, M.A. Vos (2014). The electromechanical window is no better than QT prolongation to assess risk of Torsade de Pointes in the complete atrioventricular block model in dogs. Br J Pharmacol 171(3): 714-722.

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