Pharmacologic Control of Beat-to-Beat Variability of Repolarization

to suppress and prevent dofetilide-induced Torsades de Pointes in the anesthetized CAVB dog

ISBN 978-90-3935183-3

Cover illustration: consecutive superimposed traces of endocardial MAP recordings
Layout: Scriptura, Westbroek, The Nederlands
Print: Gildeprint Drukkerijen, The Nederlands

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PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Utrecht, op gezag van de rector magnificus, prof. dr. J.C. Stoof, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op vrijdag 6 november 2009 om 16:15 door

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Financial support by the Netherlands Heart Foundation and by the Heart & Lung Foundation Utrecht is gratefully acknowledged.

Content

"Prevention is better than cure." Desiderius Erasmus (1466-1536)

Voor Imkje, Simon en de baby

Chapter 1

General Introduction

Polymorphic ventricular tachyarrhythmias are feared heart rhythm disorders as they can manifest as syncope and can quickly degenerate into ventricular fibrillation and sudden cardiac death [1-3]. A typical, well known example is drug induced Torsade de Pointes arrhythmias (TdP). This adverse effect of (new) drugs on repolarization (prolongation of QT) is an important issue for safety pharmacology. Anti-arrhythmic strategies against these life threatening rhythm disorders are still an emergent field in cardiology. Currently only two anti-arrhythmic approaches are generally accepted: 1) increasing heart rate by pacing and or isoprenaline application or 2) infusion of anti-arrhythmic agent $MgSO_4$ [4]. To better understand the arrhythmic mechanisms involved and to develop innovative approaches in handling these polymorphic ventricular arrhythmias, experimental animal models were developed.

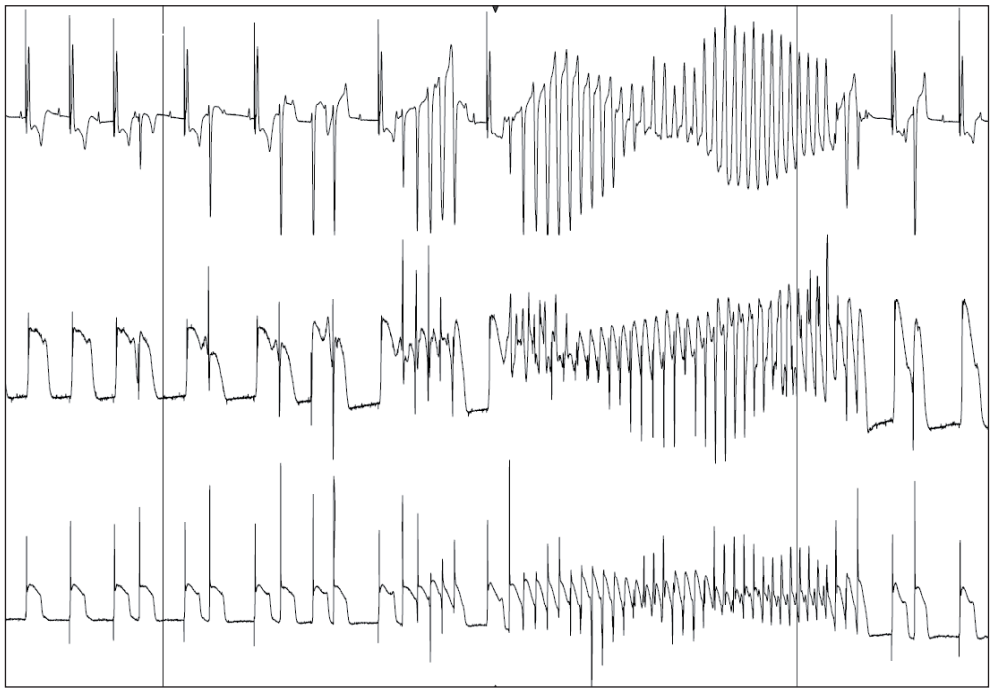
In this thesis, the dog with experimentally acquired complete atrio-ventricular block (CAVB) was once again used because of its high sensitivity for drug-induced TdP. All experiments were performed under general anesthesia. The first study (**chapter 2**) reviews the ventricular adaptations (remodeling) that occur in this animal model, with special emphasis on the electrophysiological parameters that reflect its vulnerability to arrhythmias. Beside the drug induced long-QT syndrome and TdP, this model is also known for spontaneous polymorphic ventricular arrhythmias that can lead to sudden cardiac death [5, 6]. Ventricular remodeling seen in this dog model may also resemble adaptations that occur in hearts of patients trying to compensate for similar negative stimuli of several diseases (e.g. volume overload induced hypertrophy).

For decades the duration of repolarization, measured on a regular ECG as the QT parameter, is used to estimate the risk for such polymorphic ventricular arrhythmias. However its sensitivity and specificity is rather modest [7-10]. Therefore, alternative parameters have been explored. One of them, beat-to-beat variability of repolarization (BVR) is a parameter that characterizes temporal dispersion or the lability of repolarization. In a normal heart the variation of repolarization duration on a beat to beat basis is incredibly constant and extremely low (less than 1 millisecond). The BVR methodology and its superior value compared to QT-time in predicting the occurrence of drug-induced TdP is also part of the content of **chapters 2 and 3**. In the latter chapter, also a role for BVR in estimating repolarization reserve is described.

Using the pro-arrhythmic nature of the dog with CAVB, this model is ideal to study the anti-arrhythmic effects of several pharmacological tools. In order of appearance, we have investigated AVE 0118, a novel compound with atrial specificity used as a presumably negative control (**chapter 4**), the calcium antagonists flunariziane and verapamil (**chapter 5**), the sodium channel blockers ranolazine and lidocaine (**chapter 6**) and K201, a drug with a unique effect on stabilizing the release of calcium from the Ryanodine receptor (**chapter 7**). Their anti-arrhythmic properties were tested both when the TdP was present (suppression) as well as prior to its occurrence (prevention). In addition, the capacity of the drugs to control BVR was studied: by lowering an increased BVR and/or by stabilizing BVR, the anti-arrhythmic effect of the drug could be monitored.

In all experiments, dofetilide was the drug that initiated TdP. This drug blocks the rapid component of the delayed rectifier K+ current (I_{Kr}), a property which is shared by many of the anti-arrhythmic drugs tested in this thesis. By adding additional blocking effects these drugs can not only protect for the I_{Kr} related pro-arrhythmic potential, but also offer anti-arrhythmic efficacy against dofetilide induced TdP.

Finally, for some of the drugs, their mode of action was explored in more detail. In **chapter 8**, the data have been summarized and integrated in a concept that could guide further development strategies for new antiarrhythmics.



Example of dofetilide-induced TdP in an anesthetized CAVB dog *(h603210-2)*, shown in lead II ECG and 2 monophasic action potentials recorded in the right and left ventricle (LV MAP and RV MAP).

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Chapter 2

The canine model with chronic, complete atrio-ventricular block

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Pharmacology & Therapeutics 119 (2008) 168–178

Abstract

Proarrhythmic susceptibility to drug-induced Torsades de Pointes is restricted to individuals with a predisposed phenotype characterized by a reduced repolarization reserve. Additional factors are often involved in a further impairment of repolarization, possibly culminating with dangerous ventricular polymorphic tachyarrhythmias. Drugs that block repolarizing currents represent such an additional hit.

The dog model with chronic, complete atrio-ventricular block has been used frequently for proarrhythmic drug screening. The ventricular remodeling seen after ablation of the AV node enhances the susceptibility for repolarization dependent arrhythmias. In this review, we 1) describe the cellular and molecular basis of ventricular remodeling, 2) validate the CAVB dog as a drug screening model and 3) introduce a new surrogate predictive proarrhythmic parameter: beat-to-beat variability of repolarization.

Introduction

Several publications point to the high incidence of ventricular arrhythmias and sudden cardiac death (SCD) in heart disorders associated with myocardial hypertrophy [1-5]. Polymorphic ventricular tachycardias (PVTs) are without question dangerous as they occur unexpectedly and can degenerate into ventricular fibrillation (VF) [6-8]. PVTs can occur due to various heart disorders, such as congenital long QT syndromes (LQTs): up till now there are more than 300 different mutations known in various ion channels responsible for LQTs. In each congenital syndrome, the repolarization abnormality is caused most frequently by a dysfunction in a single ion current e.g. I_{Ks} in LQT1 and I_{Kr} in LQT2 or a persistent late I_{Na} in LQT3. Less explored are the acquired LQTs as detected in phenotypes with pathologic overload of the heart resulting in ventricular hypertrophy [9, 10] and/or heart failure[11]. Relatively new are the cathecolaminergic polymorphic ventricular tachycardias (CPVT) in structurally and electrophysiologically normal hearts but with dysfunctional calcium cycling due to mutations in genes, encoding for functional proteins of the sarcoplasmic reticulum[12-15]. These facts underline the intricate importance of both electrical function and excitation-contraction coupling in ventricular arrhythmogenesis.

To comprehend and study PVTs, animal models are imperative and of great value. For almost a century, the chronic, complete atrio-ventricular block (CAVB) dog has been used as an experimental model[16]. However, just in the last decades, research attention has shifted to its use for proarrhythmic screening of drugs. Its enhanced susceptibility for (drug-induced) Torsades de Pointes (TdP) makes it an ideal model. TdP is a feared PVT characterized by a twisting shape of QRS complexes and T waves around the isoelectric line of the ECG. TdP is often described in a setting of prolonged QT interval as an adverse reaction of various pharmaceutical compounds with class-III effects, although the first TdP published was recorded in the absence of drugs in a patient with complete AV block and bradycardia[17]. In recent years, several drugs have been withdrawn from the market due to drug-induced QT prolongation and reported TdP[18, 19]. Nevertheless, drug-induced TdP is a rare arrhythmia with an incidence of less than 1 case in 10,000 to 100,000 exposures[18, 20]. Hence, if we would simply appeal to "our inside Sherlock Holmes", the first suspect in the evaluation of the proarrhythmic risk for a given therapeutic dose of a drug, would be the individual predisposition. Additionally, it became evident that other factors disturbing the repolarization process increase the heart vulnerability for ventricular arrhythmias.

The concept of repolarization reserve comprises and explains the individual differences in the proarrhythmic outcome. Repolarization reserve was initially defined as a complex of multiple mechanisms to achieve normal repolarization[21]. In healthy hearts, repolarization reserve is not impaired by the pharmacological block of one type of outward potassium current, as the other repolarizing currents may compensate and control the repolarization[22]. In other words, there is a redundancy in currents responsible for the repolarization process, prohibiting adverse effects of a single channel block. But when challenged with multiple hits, repolarization reserve can be reduced to such an extent that it becomes inadequate with the probability to culminate in potentially lethal PVTs, such as TdP[22-27]. In fact, the repolarization reserve is the ability of the heart to withstand one or more arrhythmogenic challenges [28]. The latter also includes vari-

ous heart diseases in which complex remodeling processes decrease the repolarization reserve, reflected in a vulnerability to repolarization-dependent ventricular arrhythmias. Therefore the choice for animal models to detect proarrhythmic properties of drugs should consider this predisposition, mimicking the vulnerable patient. In addition, the tested drug should be administered in doses relevant to its therapeutic plasma concentration to determine its proarrhythmic risk. When one considers not the drug but the predisposed individual as the culprit, alternative techniques could be developed to detect the susceptible patients for ventricular arrhythmias. This identification could exclude them from receiving drugs with proarrhythmic properties.

Regarding the cardiac safety assessment of drugs, two current guidelines were adopted in 2005 by the regulatory bodies of the European Union and United States. These guidelines assign both a pre-clinical strategy (ICH-S7B, 2005) as a clinical approach (ICH-E14, 2005, www.ich.org). The preclinical guidelines describe an integrated risk evaluation of a compound to delay ventricular repolarization using four levels of approach: ion channel assay, action-potential parameters, electrocardiogram (ECG) parameters and proarrhythmic effects. Still in this approach the importance of the phenotype of the animal model has been ignored.

Thus, we will discuss in this review the importance of the phenotype, in particular the CAVB dog model. Furthermore, we will introduce an electrophysiological parameter that characterizes both the vulnerable phenotype and predicts drug-induced TdP: beat-to-beat variability of repolarization (BVR). Finally, we will assess the validity of the model in proarrhythmic drug screening.

Proarrhythmic susceptibility: reduced repolarization reserve in the CAVB dog

Remodeling in the CAVB dog

There are several techniques to ablate the atrio-ventricular (AV) node: 1) injection with formaldehyde (37%) into the AV node region, 2) direct current shock, 3) clamping or crushing the region of the AV node, 4) ligation or section of the His bundle, 5) heating the area by radio-frequency (RF ablation) or 6) freezing the AV node (cryo-ablation). In the last years, the preference turned to minimal invasive transvenous approaches using catheter-delivered RF energy to induce a third degree AV-block[29].

By ablation of the AV node, the ventricular rate drops from roughly 115 to 40-50 beats/min. In the early hours there is competition between foci for dominancy. The acute bradycardia produces volume overload, leading to an increase in left ventricle end-diastolic pressure (from 9±4 to 16±4 mmHg, pooled data from de Groot et al.[30] and Donker et al.[31]) resulting in increased wall strain and increased diastolic wall stress[30-33]. Despite extensive acute neuro-humoral activation in the acute phase, there is a decrease in cardiac output of approximately 40%[31]. Still, most individuals can survive this acute situation: mortality in this stage being around 2%. In another 2% of the dogs, implantation of a pacemaker is required during the first week due to extreme bradycardia causing acute heart failure (retrospective analysis from our group).

In the days and weeks following ablation, long term adaptations occur, also called ventricular remodeling. Remodeling is well studied in this animal model and consists of a complex set of changes in structure (hypertrophy), contractility (increased dP/dt max and Ca^{2+} content of the sarcoplasmic reticulum) and electrophysiological properties of the myocardium (e.g. prolonged duration of repolarization). For a detailed list of these changes we have summarized the data in Tables 1A, 1B and in Figure 1.

Ventricular remodeling in the CAVB dog is a rapid process. The exact time frame is however difficult to address, but it is clear that electrical and hemodynamic remodeling develop relatively fast: within 2 weeks they reach their maximum[31,34]. This temporal behavior of different remodeling processes in CAVB can be seen in Figure 2. Structural remodeling, in particular left ventricular (LV) hypertrophy, seems to follow a slower path reaching completion around 4-6 weeks[35], although there is some evidence that it even further increases in time[36]. Contractility (dP/dt_{max}) starts to decline after two weeks returning to control level at 16 weeks[31]. Electrical remodeling and arrhythmogenic consequences (drug-induced TdP) are ascertained after 2 weeks and remain constant in time (Figure 2). Even up to six months, these drug-induced TdP arrhythmias were still reproducibly seen[34,37]. In most proarrhythmic studies conducted in our group, the experiments take place between weeks 3 and 9.

Myocardial stress and strain are primary mechanical stimuli for hypertrophic remodeling[31,38]. In the early days and weeks after ablating the AV node, several hormones and enzymes have been found to be increased, including norepinephrine, angiotensin II, atrial and brain natriuretic peptides. However, these humoral factors return to control levels after about six weeks[30,34,39-41].

Contractile adaptation of the two ventricles in the CAVB is relatively similar (Figure 1, left upper panel). Both at the level of ventricular myocytes (Table 1) as in the whole heart, the contractile remodeling is accompanied by a different behavior in structural and electrical remodeling: the increase in the heart weight/size is relatively larger for the right ventricle (RV) whereas electrophysiologically, the larger action potential duration (APD) are found in the LV (Figure 1). This suggests that remodeling processes are regulated also by local stimuli and/or modified by specific local factors. On the other hand, the different temporal behavior of the remodeling suggests involvement of different signal transduction pathways (Figure 2).

Ventricular remodeling and arrhythmogenic consequences

Already in the beginning of the last century[16], SCD in CAVB dogs has been reported. Turina et al. observed 3 sudden deaths in a longstanding group of 13 CAVB dogs[42]. Our group experience in more than 200 CAVB dogs, revealed an incidence of SCD in order of 10%. This prevalence has been reported under conscious conditions, free of drugs or other interventions. Using telemetry, it was possible to document the cause of death in two individuals: the onset of arrhythmias resembled the starting sequence of a TdP[3].

By challenging the dogs with clinically identified proarrhythmic drugs, TdP occur only in the remodeled phenotype, CAVB[33, 43-45]. TdP incidence in the CAVB model often exceeds 70% e.g. using class III drugs like dofetilide, azimilide or almokalant[25, 33, 46-48]. Dofetilide has

Table 1A: Remodeling depicted as changes in the CAVB phenotype (≥ 3 weeks) compared to normal dogs

Parameter	Change	Observations	References
Rhythm	**IVR**	Idioventricular; regular	
Heart rate variation	≈	adrenergic control	16, 31, 35, 39, 102
Ventricular rate	↓		
Atrial rate	≈		
Contractile remodeling			
dP/dt	↑		30, 31, 33
dP/dt max	↑		
Myocytes: resting $[Ca^{2+}]_i$	≈		54
SR Ca^{2+} content	↑		
Hemodynamic changes			
Cardiac output	≈		
Stroke volume	↑		
Fractional shortening	↑		
LV ejection fraction	↑		
Syst. blood pres.- mean	≈		30, 31, 35, 39
-systolic	≈/↑		
-dyastolic	≈/↑		
Pulmonary blood pressure-mean	≈		
-systolic	↑		
-diastolic	≈		
Vascular resistance	↑	Both systemic and pulmonary but not coronary	39, 103
Structural remodeling			
Heart weight/body weight	↑		
LV mass	↑		
RV mass	↑		
LV wall thickness and diameter	↑		
RV wall thickness/ diam.	↑	echographic,	31, 33, 35, 36, 39, 44, 50,
Left atrial diameter	↑	MRI studies	53, 104
Inferior vena cava diam.	↑		
Ventricular myocytes			
-length	↑	In RV: +23%, LV: +13%	
-width	≈		
Fibrosis	≈	Collagen staining	33, 36

Table 1B: Remodeling depicted as changes in the CAVB phenotype, compared to normal dogs (≥ 3 weeks)

Parameter	Change	Observations	References
Electrical remodeling			
PP	≈		
QRS	≈		
$QT_{(C)}$	↑		
LV MAPD	↑		3, 30, 33, 34, 36, 46, 73, 95, 104-107
RV MAPD	↑		
RV ERP	↑		
APD heterogeneities	↑	Interventricular, transseptal, transmural, apex-base	
Beat-to-beat variability of repolarization duration (BVR)	↑	LV>RV in CAVB More in SCD sub-phenotype	43, 47, 98
Cellular APD	↑	LV: +29% RV: +9%	53
Ionic remodeling			
	LV **RV**		
I_{Na+}	↓ ≈		108
I_{to}	≈ ≈		
I_{Kr}	≈/↓ ↓	LV: -15%; RV: -50%	
I_{Ks}	↓ ↓	LV: -50%; RV: -60% Also KCNQ1 and KCNE1 are reduced	26, 41, 109
I_{K1}	≈ ≈		
$ICa^{2+}_{,L\text{-type}}$	≈ ≈		54
$ICa^{2+}_{,L\text{-type}}$ window current	↑		66
Na^+/Ca^{2+} exchanger, forward	↑ ↑	LV>RV	
Na^+/Ca^{2+} exchanger, reverse	↑ ↑	LV>RV	54
$[Ca^{2+}]i$ resting	≈ ≈		
Amplitude $[Ca^{2+}]i$ transient	↑ ↑	LV: +33%; RV: +28%	
Na^+/K^+ pump	≈		65
[Na+]i subsarcolemmal	↑ ?		

Legend Table 1: ≈, similar to sinus rhythm phenotype; ↑,↓, significant increase or decrease; >, more; % represents an overall change in the mean

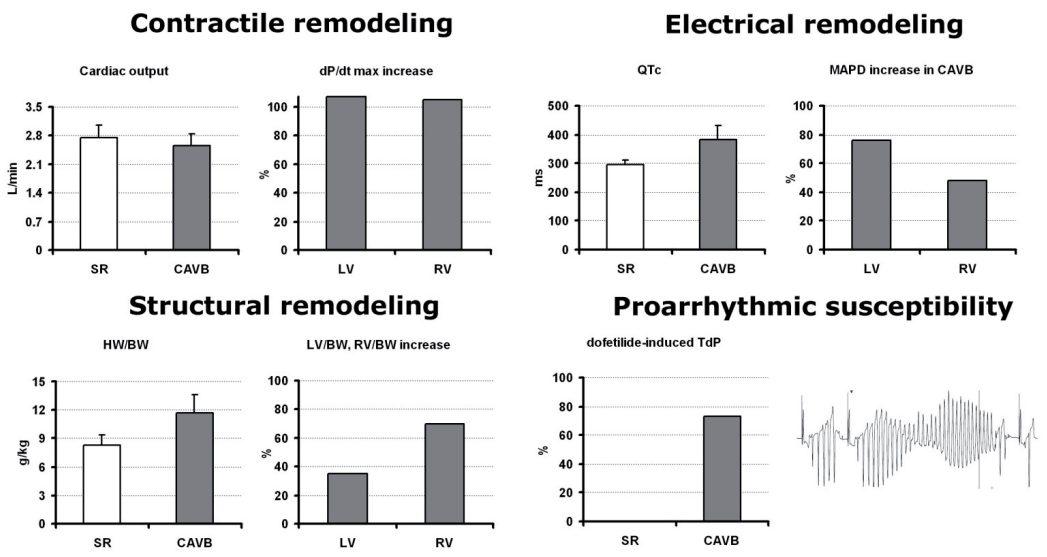

Figure 1. Ventricular remodeling in the chronic complete AV block dog model including the relative differences that occur between the ventricles (LV and RV). Contractile remodeling (left upper) shows a compensated cardiac output due to an increase in dP/dt_{max}, which is similar for both ventricles. Structural remodeling (left below) is shown as an increase in heart weight/body weight index (HW/BW) with a relative larger increase in the right ventricle. Electrical remodeling (right upper) is pictured as an increase in QTc and MAPD but now with a relative larger increase in the LV. These remodeling processes make the heart more susceptible for drug-induced TdP (right under). The incidence increases from 0% in SR to 76% in CAVB dogs.

The differences shown here between LV and RV are the overall change in the mean. Contractile remodeling data are from de Groot et al.[30], whereas for structural remodeling we used data from our own records (n=41 sinus rhythm dogs and n=111 CAVB dogs). Dofetilide-induced TdP arrhythmia incidence was gathered retrospectively too (18 individual in SR and 72 dogs in CAVB). For electrical remodeling, QTc data from the several published studies [28, 43, 47, 68, 96, 98, 105] and the difference LV vs. RV [34, 47] were pooled.

become the proarrhythmic gold standard to which new compounds are serially compared. In a large series of CAVB dogs, inducibility with dofetilide is about 76% (Figure 1). Interestingly enough, this proarrhythmic susceptibility is not present in all individuals and therefore on the basis of their outcome, CAVB dogs can be subdivided in three phenotypes: 1) sudden cardiac death, SCD phenotype, 2) drug-susceptible (to TdP) under anesthetic conditions, CAVBs (65-75% of the tested individuals) and 3) drug-resistant, CAVBr phenotype (non-responders)[40].

The underlying electrophysiological mechanisms of TdP are still discussed, although there is enough evidence for a dominant role for focal activity[49-52]. The fact that TdP, despite

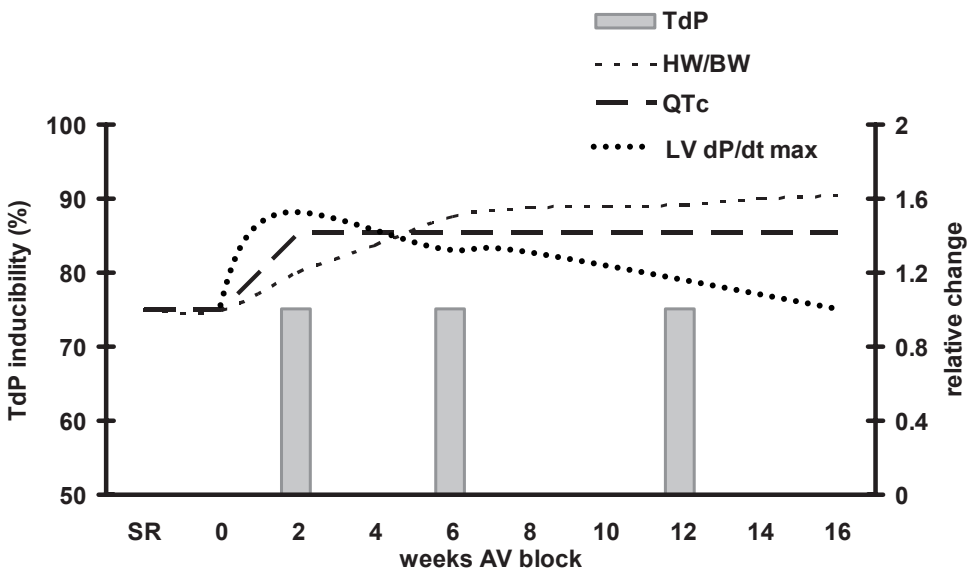

Figure 2. On the primary y axis the incidence of drug-induced TdP (% inducible individuals) is plotted on various time points, including the normal sinus rhythm (SR), right after the ablation of AV node (0 weeks) and during CAVB. On the secondary y axis, ventricular changes in the structure, heart weight/body weight index (HW/BW), contractile remodeling (dP/dt max) and electrical remodeling reflected by QT corrected for the heart rate (QTc) are plotted. One can observe 1) that TdP inducibility is present only during CAVB and is highly reproducible in time, 2) a relatively constant electrical remodeling (QTc) in time, reaching a maximum at 2 weeks, 3) contractile remodeling (an increase in LV dP/dt max) reaches a peak at 2 weeks and declines afterwards and 4) a slow increase in time of the HW/BW.
This graphic contains reference data [31, 33-36, 68].

being a fast arrhythmia, infrequently degenerates into VF and hardly ever organizes into monomorphic VT is rather compatible with focal or cellular origin. However, reentrant mechanisms could be relevant for perpetuation of the PVTs or for degeneration of TdP into VF. At the cellular level, the role of early after depolarizations (EADs) and delayed afterdepolarizations (DADs) in the initiation of triggered arrhythmias have been well documented[26,46,53,54]. An increased Ca^{2+} content of the sarcoplasmic reticulum has often been linked to triggered arrhythmias, in particular with DADs. A diastolic spontaneous calcium release from an overloaded SR produces a Ca^{2+} wave that propagates through the cell and activates a transient inward current resulting in a DAD[55]. In the CAVB dog model there is indeed an increased Ca^{2+} content and Ca^{2+} release of the sarcoplasmic reticulum. The adjustment in myocardial Ca^{2+} homeostasis leads to an enhanced susceptibility for DADs and ouabain-induced VT[30]. Successful antiarrhythmic interventions targeting calcium cycling underscore

this relationship[56-58]. On the other side, by affecting ventricular repolarization, an unbalance between inward and outward ion currents can result in EADs. Reactivation of L-type Ca^{2+} current or Na^{+} current underlies the upstroke of EADs[59,60]. Similar in the CAVB phenotype down-regulation of repolarizing potassium currents I_{Kr} and I_{Ks}, increased activity of Na^{+}/Ca^{2+} exchanger in both directions (forward and reverse), a larger window of L-type Ca^{2+} current and an enhanced dynamic modulation by SR Ca^{2+} release may contribute to an increased incidence of EADs[26,33,53,54,61-66].

Decreased repolarization reserve and requirement for additional hits

As already mentioned above, repolarization reserve is the ability of the heart to withstand one or more arrhythmogenic challenge[28]. There are several risk factors known to increase the susceptibility for TdPs: drugs, gender hormones, electrolytes disturbances (e.g. hypokalemia, hypomagnesaemia) and an increased sympathetic activity are few examples. These factors have a direct (e.g. hypokalemia, drugs, adrenergic stimulation) or indirect (e.g. increased adrenergic drive) effect on repolarization. The indirect outcome may imply changes in excitation-contraction coupling, which is intricately connected with the repolarization process and further predisposes the heart to arrhythmias[67-73].

Our group has extensive experience with the anesthetized CAVB model. Evidence that anesthesia is of relevance comes from comparisons with conscious CAVB dog studies, showing a much lower incidence of drug-induced TdPs. The group of Weissenburger had to include hypokalemia, more bradycardia and/or pacing to induce TdP after i.v. therapeutic drug infusions[74-77]. Also the group of Sugiyama and Hashimoto in Japan, has shown reproducible ventricular arrhythmias on Holter recordings in awake CAVB dogs after the treatment with a number of orally administered drugs, including cisapride[44], semitalide[78], sparfloxacin[79] and sulpiride[80]. In therapeutic concentrations, their TdP incidence is rather low.

Thus, in the CAVB model, anesthesia represents an additional hit on repolarization: it behaves like a tuner, bringing a predisposed phenotype closer to a proarrhythmic threshold. In Utrecht, anesthesia is induced with an intravenously administered barbiturate (mainly pentobarbital in our studies) and maintained by halothane in a mixture of O_2 and N_2O through the respiration-machine. Recently, due to regulatory changes halothane was replaced by isoflurane. These anesthetic regimens are all known to block repolarizing currents[81-83].

The CAVB dog model has its clinical counterparts. Several reports emerged describing the proarrhythmic risk of acquired AV-block in patients[84-87] whether occurring as a part of a pathology or after ablation of the AV node[88]. The patients with a longer QTc are predominantly female (70%)[86,87]. There are also congenital forms of complete AV-block with an incidence of 1 in 15000[89-91]. In this population remodeling may occur before the pacemaker implantation and the risk for TdP may remain in this population[89].

Quantification of repolarization reserve

In different proarrhythmic models, surrogate parameters for drug-induced alterations in ventricular repolarization and TdP have been reviewed recently[92]. Still, the electrophysiolo-

gical parameters studied in the anesthetized CAVB phenotype to predict drug-induced TdP ought to be mentioned. From a regular ECG or from MAP catheters recordings, several parameters emerged in order to characterize the electrophysiology of the heart and related proarrhythmia.

Duration of repolarization and interventricular dispersion of repolarization duration

TdP is known to occur in a setting of prolonged repolarization duration. The QT interval from a regular ECG is a parameter to measure overall cardiac repolarization duration as complex summation of vectors. Similar but invasive, the repolarization duration can be measured from local monophasic action potential (MAPs) catheters. The difference between LV and RV MAP duration is defined as interventricular dispersion (ΔMAPD). At baseline under anesthesia, repolarization duration ($QT_{(c)}$ interval) and ΔMAPD seem to be the highest in the SCD-CAVB phenotype but they cannot discriminate between drug-susceptible and drug-resistant canines[3,43].

There is an association between the blocking effect of a drug on I_{Kr} and the observed prolongation of the MAPD/QT. However, this relationship is lost when a drug has a complex action that includes effects against other ion channels[20,22,93,94]. Drug-induced prolongation of QT interval does not result in a similar TdP incidence[28,47,48,95]. Even more, QT can be prolonged by agents that do not induce TdP[95,96]. As concluded by an independent academic task force the sensitivity and specificity of the repolarization duration in predicting the proarrhythmic outcome is limited in both clinical and experimental settings. QT prolongation by itself is not a strong evidence of a genuine risk of TdP[20].

Variability of repolarization duration and drug-induced Torsades de Pointes

Beat to beat variability of repolarization (BVR) is the electrophysiological concept of changes in the repolarization duration on a consecutiveness basis. Using Poincaré plots, the repolarization duration of 30 consecutive and regular beats is plotted against the duration of the previous beats (Figure 3A). From this plot, BVR can be quantified as the area of the plot or as the mean orthogonal distance to the line-of-identity. The latter quantification is the one we preferably use and it is termed short-term variability, $STV=\Sigma|D_{n+1}-D_n|/[30x\sqrt{2}]$ where D is duration of repolarization and n is beat 1 to beat 30[28,97].

BVR is increased in the remodeled CAVB dog[43,47] and the highest in the proarrhythmic CAVB phenotypes: SCD or drug-susceptible animals[43,98]. When challenged with a drug, BVR acutely increases further prior to TdP. This increase is restricted to the proarrhythmic phenotype (Figure 3B). Furthermore, successful suppressive antiarrhythmic interventions were linked to shortening of drug-increased BVR. Increasing K^+ plasma level prevented an increase in BVR and drug-induced TdP[68]. BVR is also visible at the cellular level: it acutely increases before drug-induced EADs[28].

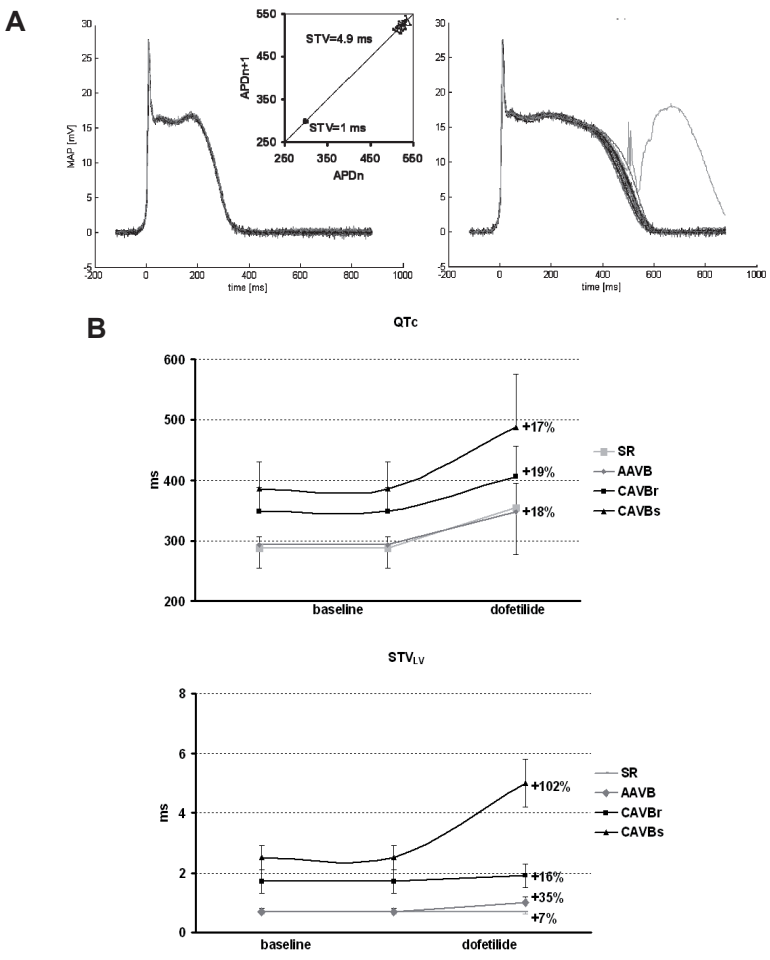

Figure 3.

A. Registration of 30 consecutive monophasic action potentials obtained from the LV endocardium in a CAVB dog (dog nr.541290-2), in baseline (left) and after adding the proarrhythmic drug dofetilide (right). In the proarrhythmic situation the beats were recorded just before the first ectopic beat, which preceded several TdP. In the small graphic, these 30 consecutive beats are shown in a Poincaré plot, the short term variability of repolarization (STV) being the average distance from the each point to diagonal. STV increased from 1.0 in baseline to 4.9 ms after dofetilide. Picture made with the courtesy of P. Oosterhoff.

B. The behavior of QTc (top) and short term variability (bottom) of the left ventricle monophasic action potential duration (STV_{LV}) in three phenotypes: 1) sinus rhythm (SR), 2) acute complete AV block (AAVB) and 3) in chronic complete AV block (CAVB). When challenged with dofetilide, TdP arrhythmias were induced in majority of the CAVB dogs (74%) but not in SR and AAVB. On this basis the CAVB group is divided in those susceptible (CAVBs) and those resistant to TdP (CAVBr). Whereas the QTc increases in all phenotypes, the elevation of STV after dofetilide is restricted to TdP-susceptible CAVB dogs.

Data published in Thomsen et al.[43].

CAVB dog as a reliable model for proarrhythmic drug screening

Proarrhythmic sequence, positive control in serial testing and repeatability of drug-induced TdP

After the administration of a drug till the actual occurrence of TdP, three phases can be frequently recognized: first repolarization duration (QT/QT_c) prolongs (general observation), then ectopic activity arises which may be followed in time by TdP (Figure 4A). Drug-induced ectopic beats are premature ventricular complexes, defined as short-coupled beats arising from a new ventricular focus before the repolarization of the previous beat is complete (Figure 4A). Both single ectopic beats (SEB) and multiple ectopic beats (MEB) can be counted for a given time interval. We define drug-induced TdP as a PVT consisting of at least five beats characterized by a twisting shape of QRS complexes and T waves around the isoelectric line of the ECG. In the past, TdP quantification was often expressed as incidence being the number of inducible individuals that show reproducible TdP, defined as more than 3 TdP per experiment. However this approach fails to include a measure of severity. To quantify the TdP gravity, the frequency of episodes (number of TdP/time interval), the duration and the rate of TdP defibrillations can be considered. The quantification of arrhythmias is relevant especially when antiarrhythmic interventions are investigated or when the proarrhythmia between drugs is compared. As an example we show the results of such quantification using dofetilide. In all anesthetized studies performed over the last four years, TdP incidence with dofetilide (n=72) was 76%. In these susceptible individuals, 13 TdP were counted on average in the first 10 minutes. Most of these TdP were self terminating, in 20% electrical conversion had to be performed as they were longer than 10 seconds or due to degeneration into VF. Additionally, in this 10 minutes interval, 50 single and 25 multiple ectopic beats were on average counted (Figure 4B). To assess the temporal behavior of this pro-arrhythmic outcome, a smaller group (n=8) was followed for another 10 minutes interval, in the absence of an antiarrhythmic intervention. In figure 5A, one can appreciate that the TdP arrhythmia further persists.

According to the definitions of National Institute of Standards and Technology, USA (www.nist.gov), repeatability over weeks of dofetilide-induced TdP was assessed in a group of 14 animals. This analysis revealed an accuracy of 95.5% and a precision of 2.3% (expressed as variance). Interestingly, not only the TdP incidence was highly reproducible but also the doses needed to induce TdP as well as the gravity of TdP arrhythmias were comparable between experiments (Figure 5B).

A sensitive and specific model for proarrhythmic drug screening

In a group of patients with heart failure, dofetilide (0.014 mg/kg daily) had a TdP incidence of 3.3%[99]. In the anesthetized CAVB dogs, a single comparable therapeutic dose (0.025 mg/kg/5 min) however had an incidence of around 76%. This increased sensitivity in the CAVB model is similar for other drugs when compared to TdP reports in patients, e.g. d-

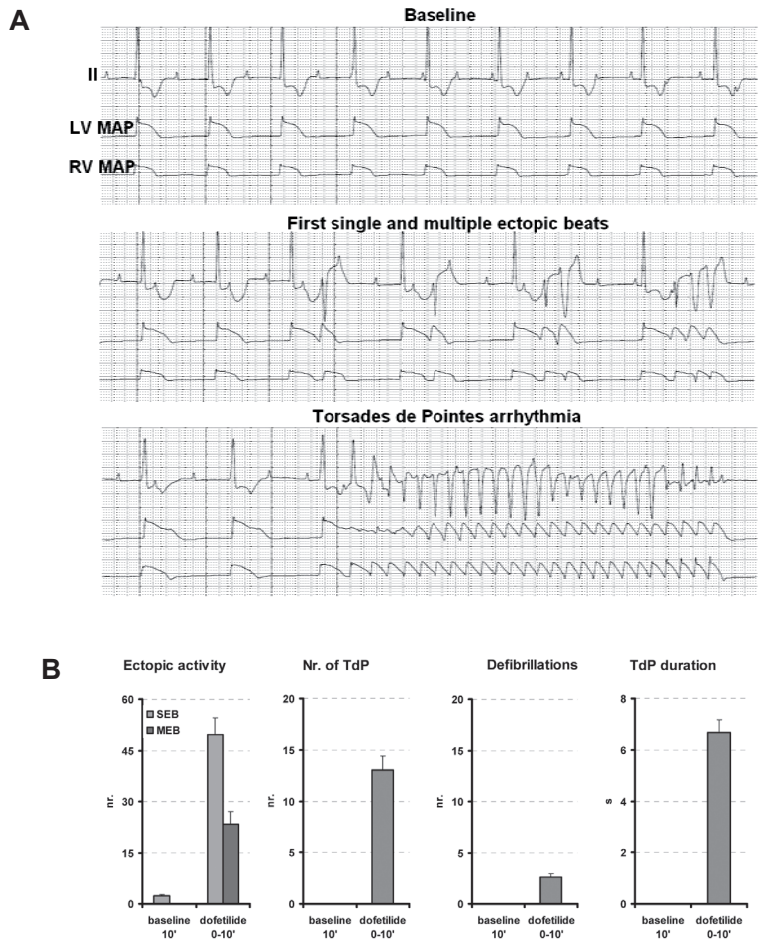

Figure 4.

A. Electrophysiological recordings (lead II ECG, LV and RV MAP, from top to bottom) illustrating the sequence of arrhythmia in an anesthetized CAVB dog during a test with dofetilide. Top panel represents the control situation. Middle part illustrates the occurrence of the first single ectopic beats (SEB) followed by multiple ectopic activity (MEB). The lower fragment shows the occurrence of the first, self terminating TdP. Recordings are shown on scale paper, at 25mm/s, 40mV/cm amplitude for LV and RV MAPs and 0.5mV/cm for ECG.

B. Quantification of dofetilide-induced arrhythmias in a large group of anesthetized CAVB dogs. The number of single and multiple ectopic beats (SEB and MEB) are shown over a 10 minutes time interval. Similar, the number of TdP is shown. In addition the severity of TdP arrhythmias, quantified as number of defibrillations and duration of TdP have been quantified. TdP were electrically converted if they were longer than 10 seconds or degenerated into VF.

Data shown as average ± standard error of the mean (n=55).

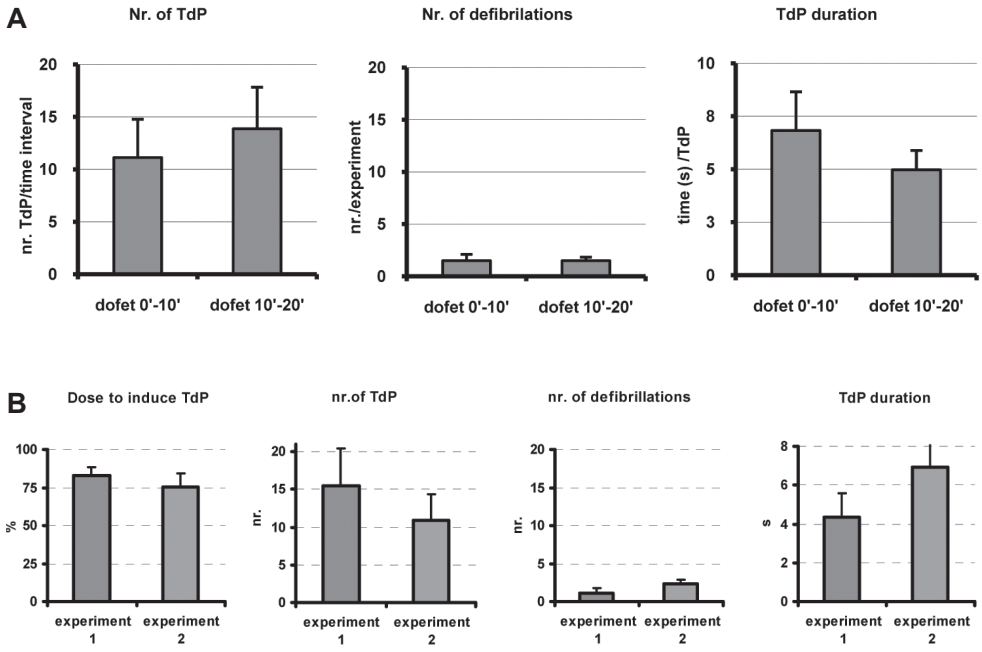

Figure 5.

A. Persistent emergence of dofetilide-induced TdP during the experiment is shown in a group of CAVB dogs. In the absence of an antiarrhythmic intervention, both the number of TdP (per 10 minutes time interval) and severity (number of defibrillation and duration of TdP) is comparable between the two '10 minutes' intervals.

Data shown as average ± standard error of the mean (n=8).

B. This figure pictures the repeatability of drug-induced arrhythmia in time. Two serial experiments in the same CAVB dogs were performed. The first graphic shows the relative dose of dofetilide needed to induce TdP. Dofetilide i.v. infusion was stopped with the first TdP (0.025 mg/kg in 5 min represents a full dosage, 100%). For both experiments the number and the severity of TdP were quantified for the first 10 minutes interval of dofetilide infusion.

Data shown as average ± standard error of the mean (n=14).

sotalol[28,100], almokalant[3,51,101], azimilide[25] and sertindole[48]. Still the CAVB dog model also recognizes safe drugs (no TdP) despite lengthening of repolarization duration. Examples are amiodarone and moxifloxacin in the anesthetized dogs[95,96] and amiodarone in conscious dogs[44,78].

This proarrhythmic sensitivity and specificity of the CAVB model is reflected by BVR not only in baseline but also in drug testing conditions. With various I_{Kr}-blockers, BVR abruptly increases before the first ectopic beat, predicting the risk for drug-induced TdP[28,43,47,68]. Safe drugs as well as safe doses did not increase BVR despite prolonging repolarization[28,95,96], Figure 6A.

As mentioned, the conscious CAVB model, detect proarrhythmia only in supra-therapeutic doses of known proarrhythmic drugs: e.g. terfenadine was proarrhythmic in 10 times higher therapeutic concentrations. However, also this proarrhythmic effect was linked to an increase in BVR, now quantified from QT interval[45]. Figure 6 summarizes these findings.

Conclusions

I. We consider the conscious or anesthetized CAVB dog as a suitable and relevant model for proarrhythmic drug screening. This model allows serial investigations, enabling the use of a positive control and considers TdP arrhythmias as an endpoint. Only the anesthetized CAVB model is appropriate to test drugs in relevant therapeutic concentration.
II. This enhanced sensitivity for repolarization dependent arrhythmias does not preclude the identification of safe drugs (high specificity).
III. The ventricular remodeling processes that occur in the CAVB dog have been well studied and explained on cellular and molecular level, in particular the reduced repolarization reserve by down regulation of ion channels (channelopathies of IKr and IKs) has been well documented.
IV. The concept of repolarization reserve can be quantified using BVR.

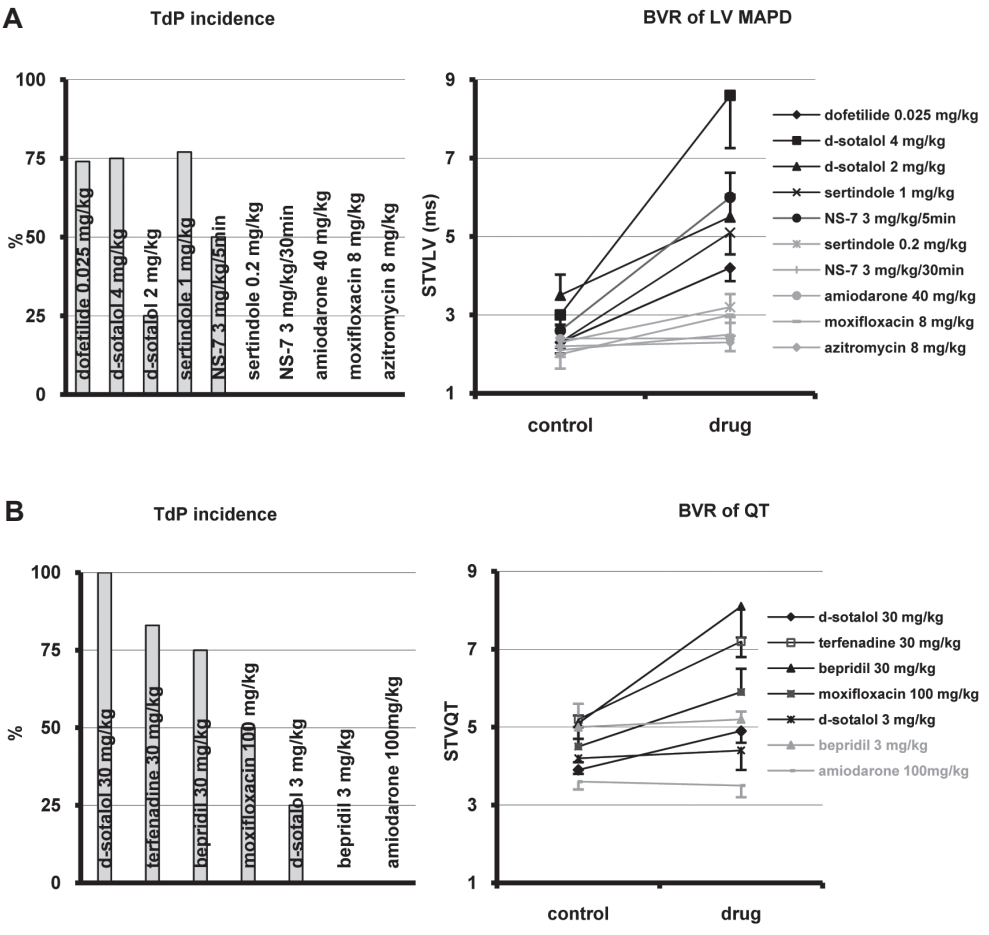

Figure 6. TdP incidence expressed as number of inducible individuals (left graph) and drug-induced changes in BVR (right graph) in several proarrhythmic drug tests in the anesthetized (A, top) and awake CAVB dog model (B, bottom). When no TdP occurred, BVR was depicted in grey. Proarrhythmic effects were assessed after a single dose in most drugs with exception of amiodarone, where 4 weeks treatment was followed. Data is shown as average ± standard error of the mean.

For the studies with anesthetized CAVB model, data was used from the following references: dofetilide [43]; d-sotalol [28]; sertindole [48, 68]; NS-7 [47]; moxifloxacin [96] amiodarone [28, 95]. For the conscious studies references are as follow: d-sotalol and amiodarone [110], terfenadine [45], bepridil [111] and moxifloxacin [96].

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Chapter 3

Proarrhythmic electrical remodelling is associated with increased beat-to-beat variability of repolarisation

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Received 3 July 2006; received in revised form 30 October 2006; accepted 20 November 2006

Cardiovascular Research 73 (2007) 521–530

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ABSTRACT

Objective: Acquired long-QT syndrome in combination with increased beat-to-beat variability of repolarisation duration (BVR) is associated with lethal Torsades de Pointes arrhythmias (TdP) in dogs with remodelled heart after complete atrio-ventricular block (AVB). We evaluated the relative contributions of bradycardia and ventricular remodelling to proarrhythmic BVR with and without pharmacological I_{Kr} block in order to identify the individual at risk.

Methods: Three groups of dogs were used: Sinus-rhythm dogs (n=12); dogs with acute AVB (AAVB, n=8); and dogs with >3 weeks chronic AVB (CAVB, n=27). Under anaesthesia, ECG and monophasic action potential duration (MAPD) were measured. Local BVR was quantified as short-term variability from 30 consecutive left ventricular MAPD ($STV=\Sigma|n_i-n_{i+1}|/[30\times\sqrt{2}]$). All dogs received dofetilide *i.v.*.

Results: The slower ventricular rate acutely after AVB neither affected QTc nor STV (288±18 to 293±38 ms and 0.7±0.1 to 0.7±0.1 ms, respectively; *P*=NS for both), whereas ventricular remodelling increased both parameters (to 376±46 and 2.3±0.6 ms, respectively; *P*<0.05 for both). Neither dogs in sinus rhythm nor acute AVB showed any TdP, whereas dofetilide induced TdP in 74% of the CAVB dogs. Dofetilide increased the QTc interval in all groups (19-24%; *P*<0.05 for all groups), whereas STV was elevated in CAVB dogs only (to 4.2±1.5 ms; *P*<0.05) and further confined to inducible-CAVB dogs (5.0±0.8 versus 1.9±0.4 ms for resistant dogs; *P*<0.05). Variability of the idioventricular rate was increased directly after AVB and did not influence BVR.

Conclusions: Under drug-free circumstances, a persistent high BVR in CAVB dogs is remodelling dependent rather than a direct consequence of bradycardia acutely after AVB. Variability of this slower rate does not influence BVR. Dofetilide causes a transient increase in BVR only in proarrhythmic dogs. Thus, BVR may aid the identification of the TdP-susceptible patient.

Introduction

Torsades de Pointes arrhythmia (TdP) is a serious ventricular polymorphic tachycardia, which may herald fatal ventricular fibrillation and sudden death. By definition, the arrhythmia is associated with a prolonged QT interval of the ECG[1]. Drug-induced TdP can be induced by various pharmacological medications, but occurs almost exclusively in patients with an underlying cardiac pathology elevating their vulnerability to repolarisation-dependent arrhythmias[2,3]. Instead of prohibiting beneficial medical treatments to avoid drug-induced TdP, identification of the vulnerable patient could be an advantageous strategy[4].

Recently, several studies have concluded that QT prolongation on its own is not a reliable predictor of drug-induced TdP, whereas alternative or additional surrogate parameters have been proposed[5-8]. We have suggested temporal beat-to-beat variability of repolarisation duration (BVR) as a candidate parameter[5]. Our research was performed in anaesthetised dogs with chronic complete atrio-ventricular block (CAVB), with a ~70% incidence in TdP provoked by class-III antiarrhythmic drugs[9,10]. This enhanced susceptibility results from ventricular remodelling following the onset of chronic bradycardia[11,12]. The electrophysiological fraction of the remodelling processes features primarily a heterogeneous prolongation of repolarisation duration manifested by QTc prolongation in vivo. Furthermore, a chronically increased BVR is observed at baseline[13]. In this model, baseline BVR has been successfully employed to identify predisposition of individual animals: those showing large BVR die suddenly in the absence of anaesthesia and drugs[13].

In the present study, we evaluate the relative contributions of bradycardia and ventricular electrical remodelling to the persistently increased baseline BVR present in anaesthetised CAVB dogs. Furthermore, we analyse the influence of physiological heart-rate variability on BVR and the response of BVR to pharmacological I_{Kr} block. Finally, we compared values of BVR in dogs with and without drug-induced proarrhythmia.

Methods

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and is in accordance with the European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EU). The local animal-care committees of Maastricht and Utrecht Universities approved all experiments.

General

Experiments from 47 mongrel dogs of either sex were included in this investigation. 12 experiments in dogs in sinus rhythm and 9 experiments in dogs in chronic AVB have been reported upon previously[9,10], whereas 11 chronic AVB dogs are part of ongoing pharmacological studies on novel experimental drugs, where dofetilide serves as reference drug for proarrhythmia. BVR has not been reported for any of these dogs previously. Generally,

inclusion criteria were: 1) anaesthesia induced by sodium pentobarbital (25 mg/kg i.v.) and maintained by 0.5% halothane in a mixture of O_2 and N_2O (1:2); 2) high quality recordings of monophasic action potentials (MAP, EP technologies, CA) from the left (LV) and right ventricle (RV); and 3) administration of 25 µg/kg dofetilide i.v over 5 minutes.

Steerable MAP recording catheters were placed under fluoroscopic guidance at the endocardium of each ventricle. Endocardial location was selected based on MAP amplitude and reproducibility. In all experiments, 6 ECG leads from the limbs and the 2 MAPs were recorded continuously. Signal processing, recording and animal care have been described in detail earlier[10]. In 35 dogs, complete AVB had been created by radiofrequency ablation as previously described[14]. Of these, 8 were studied acutely, 20-30 minutes after AV-nodal ablation, whereas the remaining 27 animals were studied after a minimum of 3 weeks, a sufficient time period for stabilisation of electrical remodelling[14].

Experimental protocol

Dofetilide was dissolved in 100 µl 0.1 M HCl and diluted in 0.9% saline to a final volume of 0.5 ml/kg bodyweight and administered over 5 minutes or until TdP appeared. The acute electrophysiological effects of dofetilide were studied for 15 minutes covering the time-points of both maximal plasma concentration[9] and maximal cardiac effect[10]. Any TdP that degenerated into ventricular fibrillation was stopped by electrical cardioversion.

Data analysis

Applying a custom-made computer programme (ECGview, Maastricht University, The Netherlands), we measured the following parameters offline at a resolution of 2 ms: RR and QT intervals from lead II and LV and RV MAP duration to 100% repolarisation (MAPD). All measurements were performed on 30 consecutive beats with the same focus of activation before and 10 minutes after the start of dofetilide administration. Due to substantial extrasystolic activity of the ventricles in chronic-AVB dogs after dofetilide, analysis was performed immediately prior to the first extra systolic beat. Interventricular dispersion of repolarisation duration (ΔMAPD) was defined as LV minus RV MAPD. Heart-rate corrected QT intervals were calculated according to van de Water's formula[15]. BVR was determined according to earlier publications[5]. Briefly, Poincaré plots were drawn from 30 consecutive LV MAPD measurements and short-term variability ($STV_{LV}=\Sigma|D_n - D_{n-1}|/[30\times\sqrt{2}]$, where D_n represents the LV MAPD of the n^{th} beat), representing the mean orthogonal distance to the line-of-identity, was calculated. Additionally, STV of the RR, PP, and QT intervals (STV_{RR}, STV_{PP}, and STV_{QT} respectively) were determined using the same algorithm. Torsades de Pointes arrhythmia (TdP) was defined as a polymorphic ventricular tachycardia of at least 5 beats with a typical twisting around the isoelectric line in the setting of a prolonged QT interval. A dog was considered inducible when 3 or more TdP occurred as a consequence of dofetilide administration. A second investigator confirmed all observations and measurements.

Statistical analysis

Pooled data are expressed as mean±SD. All comparisons of electrophysiological data were performed with a 1 or 2-way ANOVA followed by a Bonferroni *t*-test when appropriate. Inducibility was compared using Fisher's exact test. Association between pairs of variables were analysed with Pearson product moment correlation test. SigmaStat (v. 2.03; SPSS Inc.) was used for statistical analysis. Significance was set at $P<0.05$. The area under the receiver-operating characteristics was used to asses the proarrhythmic-predictive power of electrophysiological values. Furthermore, we determined the predictive power of electrophysiological parameters combined through multiplication ($A \times B$, where A and B are 2 different electrophysiological parameters).

Results

Electrophysiological parameters defining the 3 groups

As expected, the sinus rhythm group was characterised by a faster ventricular rate than the AVB groups (Table 1). In contrast, the shortest PP intervals were seen in the acute-AVB dogs. Although repolarisation duration in general showed a trend towards prolongation after AVB, this was entirely due to the slowed ventricular rate, as the QTc intervals were equal at sinus rhythm and acute AVB. Electrical remodelling after AVB quantified as QTc prolongation relative to sinus rhythm was 2±34 ms (1%; P=NS; n=8) and 95±49 ms (34%; $P<0.05$; n=27) for acute and chronic AVB, respectively. Prolonged repolarisation times at chronic AVB were also observed in the group comparison of Table 1. Representative examples of the electrophysiological parameters are given in Figure 1.

The dofetilide challenge prolonged the RR and QT intervals as well as LV and RV MAPD in all 3 groups (Table 1). The absolute increase in ventricular repolarisation duration was comparable between the 3 groups (Table 1), although maximum values were higher in chronic-AVB dogs. Torsades de pointes arrhythmia was neither observed at baseline nor after dofetilide in dogs in sinus rhythm or in acute AVB (Fig. 1), whereas the incidence of dofetilide-induced TdP in the chronic-AVB group was 74%.

Parameters of temporal dispersion in the 3 groups

Short-term variability of the LV MAPD increased as a consequence of remodelling after AVB, whereas bradycardia alone did not alter STV_{LV} (Fig. 2A). Variability of the beat-to-beat cycle length (STV_{RR}) during idioventricular rhythm was larger than when the RR interval was measured during sinus rhythm, but it was not affected by ventricular remodelling. Dofetilide caused an increase in the STV_{LV} only in the remodelled chronic-AVB dogs and not in the other 2 groups. STV_{RR} was only affected by dofetilide when the ventricles were under sinus-node control (Fig. 2A).

Table 1: Electrophysiological parameters before and after administration of 25 µg/kg dofetilide in the 3 groups of dogs

	Sinus rhythm	Acute AVB	Chronic AVB
Baseline	n=12	n=8	n=27
RR	524±83	1111±281*	1245±179*
PP	524±83	372±31*	558±105†
QT	246±24	303±34	397±54*†
QT_c	288±18	293±38	376±46*†
LV MAPD	206±15	239±22	337±41*†
RV MAPD	191±15	224±21	295±39*†
ΔMAPD	16±11	14±8	42±27*†
TdP incidence	0%	0%	0%
Dofetilide			
RR	646±127‡	1360±298*‡	1345±205*‡
PP	646±127‡	416±62*	593±98†
QT	323±51‡	379±72‡	497±74*†‡
QT_c	354±41‡	348±71‡	467±66*†‡
LV MAPD	295±53‡	324±78‡	440±58*†‡
RV MAPD	260±26‡	274±60‡	364±58*†‡
ΔMAPD	35±34	50±22‡	84±49*†‡
TdP incidence	0%	0%	74%

All values in milliseconds unless otherwise noted. TdP inducibility is quantified relative to group sizes (12, 8 and 27, respectively). *, $P<0.05$ versus sinus rhythm; †, $P<0.05$ versus acute AVB; ‡, $P<0.05$ versus baseline.

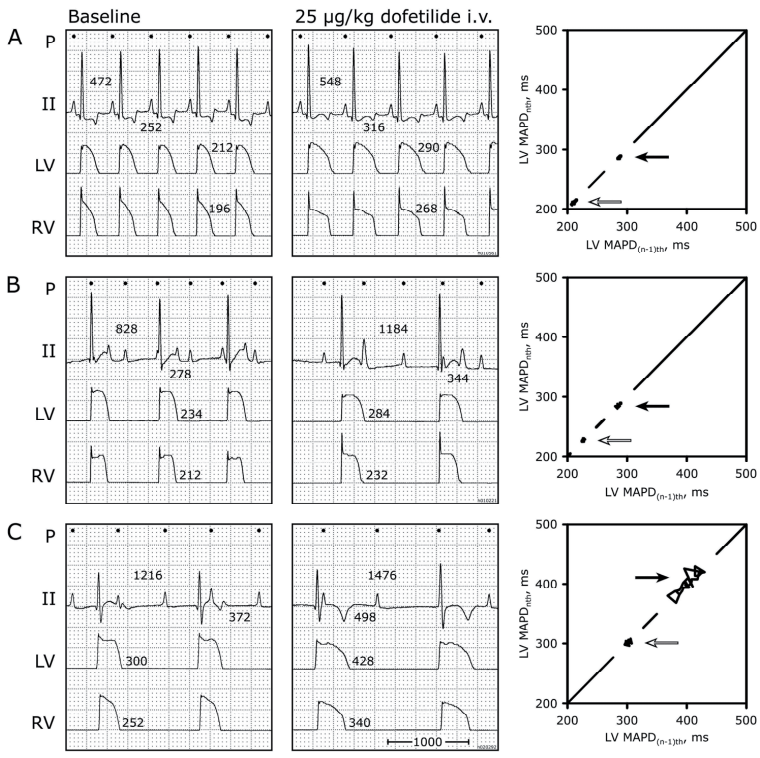

Figure 1. Electrophysiological characteristics of the 3 phenotypes used in the current study. Shown in each trace are lead II and the LV and RV MAP recordings. Measured RR intervals and QT interval are above and below the ECG, respectively. MAPD is noted adjacent to the measured action potential. All measurements are mean of 30 beats (all ms). ECG calibrated to 1 mV/cm and a paper speed of 20 mm/s (horizontal scale bar). MAP signals calibrated to 30 mV/cm. Dots above the ECG are aligned with P waves. Right-most panels are Poincaré plots depicting 30 consecutive LV MAPD from around the traces to the left. Arrows indicate Poincaré plots under the influence of dofetilide. Sinus rhythm (A) is characterised by synchrony between P waves and QRS complexes indicating normal AV-node conduction. There is no bradycardia and repolarisation duration at baseline is relatively short. Administration of dofetilide prolongs repolarisation duration but does not increase the size of the Poincaré plot. Acute AVB (B) causes prolongation of the RR interval and rate-dependent prolongation of repolarisation durations. There is complete asynchrony between P waves and QRS complexes. Dofetilide has the same effects on repolarisation duration and on the Poincaré-plot size as in sinus rhythm. At chronic AVB (C), ventricular remodelling has caused pronounced prolongation of repolarisation duration. Dofetilide causes a further increase in both the QT interval and the ventricular MAPD and increases beat-to-beat variability of repolarisation as is obvious from the Poincaré plot. TdP arrhythmias were only seen after dofetilide in dogs with chronic AVB.

In sinus rhythm, the dofetilide-induced increase in STV_{RR} (1.3±0.8 to 2.9±1.7 ms; *P*<0.05; Fig. 2), had no effect on STV_{QT} (1.0±0.2 to 1.2±0.4 ms; *P*=NS) or STV_{LV} (0.7±0.1 to 0.7±0.1 ms; *P*=NS; Fig. 2A). Representative examples of Poincaré plots are shown in Figure 3. Even when performing a beat-to-beat heart-rate correction of the QT interval, the STV of these QTc intervals were not increased (STV_{QTc}: 1.1±0.3 to 1.3±0.4 ms; *P*=NS).

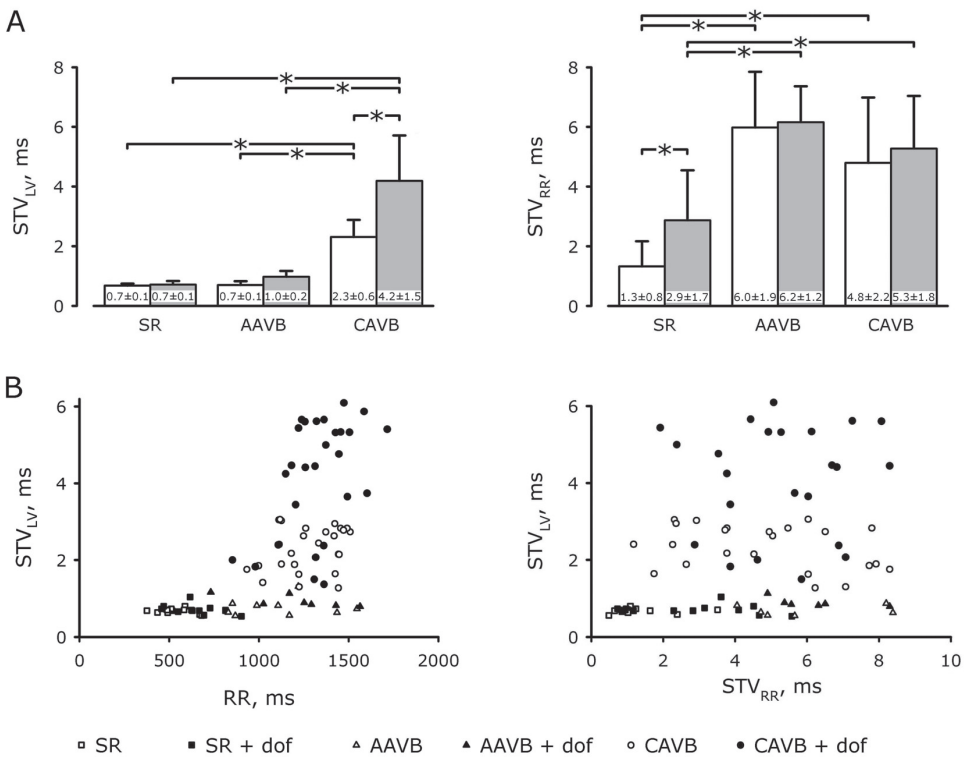

Figure 2. Temporal variability of ventricular repolarisation and rate at baseline (open bars) and after administration of 25 µg/kg dofetilide i.v. (gray bars) in anaesthetised dogs in sinus rhythm, acute (AAVB) and chronic (CAVB) AVB. At baseline, STV_{LV} is increased in the chronic AVB group, and dofetilide only elevates STV_{LV} in this group. *, *P*<0.05. Ventricular-rate variability quantified by STV_{RR} is increased after AV-node ablation, but this is not remodelling dependent. Dofetilide increases the sinus rhythm STV_{RR}, but has no effect on STV_{RR} after AVB. Actual values are noted within bars. Panel (B) shows STV_{LV} as a function of RR interval and STV_{RR} for individual dogs. There was a statistical significant correlation between the RR interval and STV_{LV} (r=0.6; *P*<0.05; n=94; left), but not between STV_{RR} and STV_{LV} (r=0.1; *P*=NS; n=94; right).

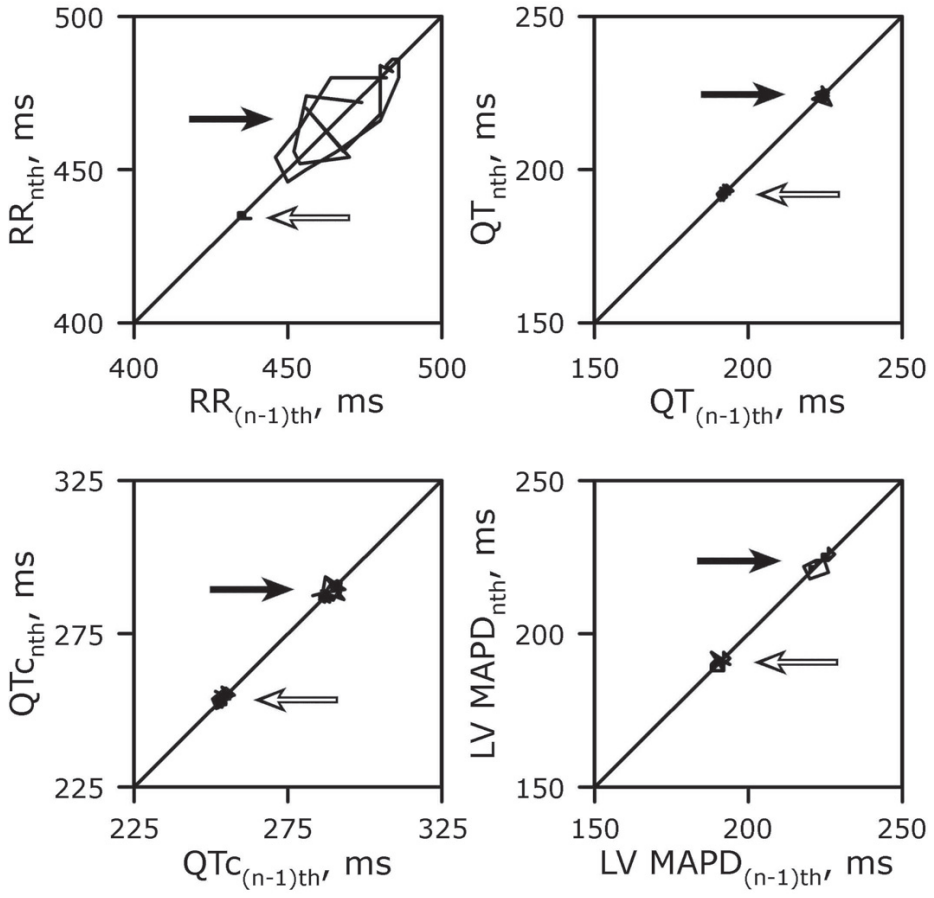

Figure 3. Four pairs of representative Poincaré plots of the RR, QT, and QTc intervals and LV MAPD for the same 30 consecutive beats in sinus rhythm. Open arrow, baseline; closed arrow, dofetilide. Only the variability of the RR interval is increased by dofetilide, whereas the QT, QTc, and LV MAPD plots are virtually unchanged.

STV_{PP} was comparable to STV_{RR} in sinus rhythm and increased with dofetilide (STV_{PP}: 2.0±0.9 to 3.4±1.9 ms; P<0.05; n=12). To evaluate the effect of ventricular bradycardia and remodelling on the atria, we determined STV_{PP}, which increased to 12.0±18 ms (P<0.05; n=27) after 3 weeks AVB. This increase was based on a heterogeneous response, as evidenced by a range of STV_{PP} between 1.5 and 71 ms (Fig. 4). Dofetilide did not significantly affect STV_{PP} after AVB (7.6±7.8 and 17.5±15 ms after acute and chronic AVB, respectively; P=NS for both).

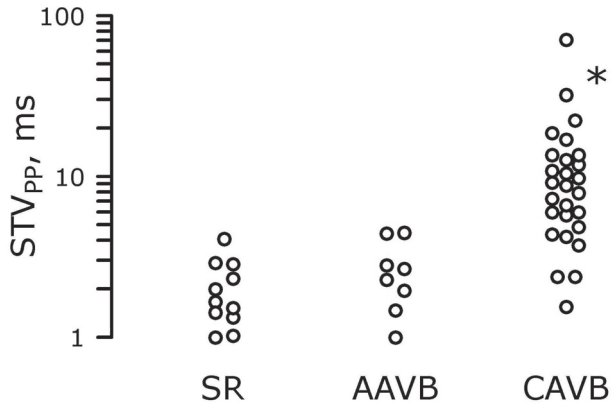

Figure 4. Beat-to-beat variability of sinus-rhythm cycle length. Under baseline conditions, STV_{PP} is increased after 3 weeks AVB, however a large inter-individual variability within the chronic-AVB dogs (CAVB) is present. Note the logarithmic STV_{PP} scale. *, *P*<0.05 versus sinus rhythm and acute AVB (AAVB).

Electrophysiological characteristics of the TdP-prone dog

Seven of the 27 dogs with chronic AVB were resistant to dofetilide-induced TdP. Compared to the inducible chronic-AVB dogs, RR and QT intervals, LV and RV MAPD and STV_{LV} were all significantly lower at baseline in the resistant dogs (Table 2). Dofetilide increased most electrophysiological parameters in both sub-groups, and both RR and QT intervals reached higher maximal values in the inducible sub-group. STV_{RR} or STV_{PP} were not affected by dofetilide, however STV_{LV} was only increased in the TdP-inducible dogs (Table 2; Figure 5).

The TdP-predictive value of the different electrophysiological parameters was determined from the chronic-AVB dogs (Table 3). This analysis was performed by calculating the area under the curve of the receiver-operating characteristics (Fig. 6). This area for STV_{LV} after dofetilide administration was 1.0 indicating total separation of STV_{LV} values. As illustrated in Figure 6, all TdP-inducible chronic-AVB dogs had an acute dofetilide-induced elevated STV_{LV} reaching values above 3.0 ms. On the other hand, all resistant dogs had values below 3.0 ms. A combination of 2 of the electrophysiological parameters by simple multiplication increased the AUC considerably in several incidences (Table 4).

Electrophysiological characteristics of the TdP-prone dog

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Table 2: Electrophysiological effects of dofetilide in chronic-AVB dogs divided into resistant and inducible dogs on the basis of dofetilide-induced TdP

	Sinus rhythm	Acute AVB	Chronic AVB
Baseline	n=12	n=8	n=27
RR	524±83	1111±281*	1245±179*
PP	524±83	372±31*	558±105†
QT	246±24	303±34	397±54*†
QT_c	288±18	293±38	376±46*†
LV MAPD	206±15	239±22	337±41*†
RV MAPD	191±15	224±21	295±39*†
ΔMAPD	16±11	14±8	42±27*†
TdP incidence	0%	0%	0%
Dofetilide			
RR	646±127‡	1360±298*‡	1345±205*‡
PP	646±127‡	416±62*	593±98†
QT	323±51‡	379±72‡	497±74*†‡
QT_c	354±41‡	348±71‡	467±66*†‡
LV MAPD	295±53‡	324±78‡	440±58*†‡
RV MAPD	260±26‡	274±60‡	364±58*†‡
ΔMAPD	35±34	50±22‡	84±49*†‡
TdP incidence	0%	0%	74%

All values in milliseconds unless otherwise noted. TdP inducibility is quantified relative to group sizes (12, 8 and 27, respectively). *, $P<0.05$ versus sinus rhythm; †, $P<0.05$ versus acute AVB; ‡, $P<0.05$ versus baseline.

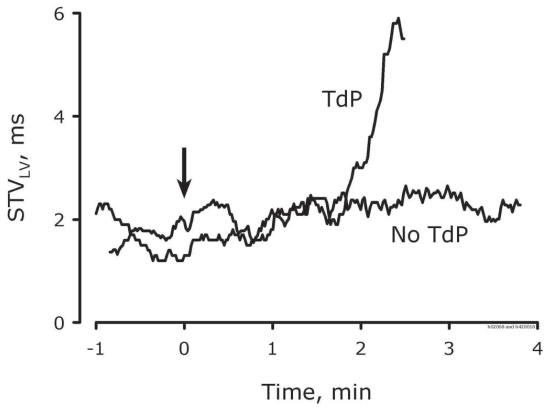

Figure 5. Temporal development of STVLV obtained as a 30-beat moving average in 2 dogs with chronic AVB. Arrow indicates start of dofetilide administration. Only the inducible dog has an instantaneous increase in STVLV as a consequence of dofetilide infusion.

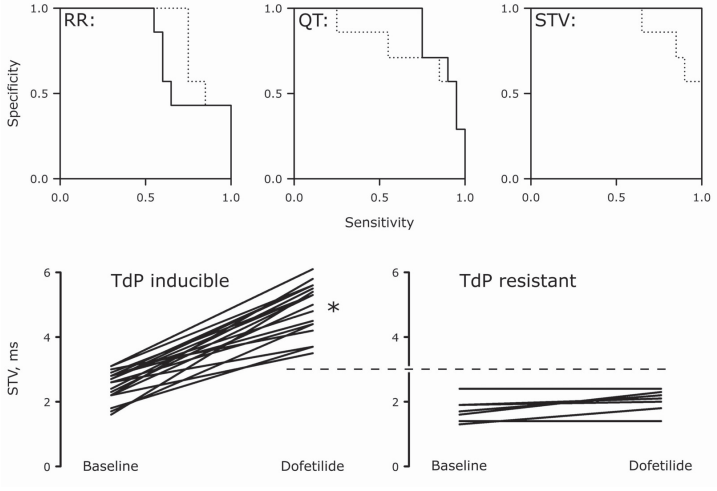

Figure 6. Proarrhythmic-predictive value of various parameters as determined by receiver-operating characteristics (ROC), plotting specificity as a function of sensitivity over a wide range of cut-off values. The area under the curve of these plots quantifies the predictive value (Table 3). Only chronic-AVB dogs are included in the analysis. ROC plots (above) for the RR and QT interval and the STVLV at baseline (dotted lines) and after dofetilide (solid lines). In this study with limited group sizes of inducible and resistant dogs with chronic AVB, there is a perfect ROC plot with an area of 1, indicating failsafe predictive value. This is due to total separation of the dofetilide-elevated STVLV in the 2 groups (below). Thus, a cut-off value of 3.0 ms (dashed line) discriminates between TdP inducible and resistant dogs in anaesthetised dogs with chronic AVB and dofetilide challenge.

most electrophysiological parameters in both sub-groups, and both RR and QT intervals reached higher maximal values in the inducible sub-group. STV_{RR} or STV_{PP} were not affected by dofetilide, however STV_{LV} was only increased in the TdP-inducible dogs (Table 2; Figure 5).

The TdP-predictive value of the different electrophysiological parameters was determined from the chronic-AVB dogs (Table 3). This analysis was performed by calculating the area under the curve of the receiver-operating characteristics (Fig. 6). This area for STV_{LV} after dofetilide administration was 1.0 indicating total separation of STV_{LV} values. As illustrated in Figure 6, all TdP-inducible chronic-AVB dogs had an acute dofetilide-induced elevated STV_{LV} reaching values above 3.0 ms. On the other hand, all resistant dogs had values below 3.0 ms. A combination of 2 of the electrophysiological parameters by simple multiplication increased the AUC considerably in several incidences (Table 4).

Table 3: Proarrhythmic predictive values of single electrophysiological parameters analysed for Table 2

	Baseline	Dofetilide
RR	0.87	0.77
PP	0.91	0.92
QT	0.79	0.90
QT_c	0.74	0.88
LV MAPD	0.86	0.74
RV MAPD	0.84	0.71
ΔMAPD	0.50	0.58
STV_{RR}*	0.71	0.51
STV_{PP}	0.73	0.68
STV_{LV}	0.91	1.00

Area under the receiver-operating characteristic plots (Fig. 6). The closer the area for a given parameter is to 1, the better its proarrhythmic-predictive performance in anaesthetised dogs with chronic AVB. *, low STV_{RR} is predictive for TdP, whereas high values for all other analysed electrophysiological parameters have proarrhythmic-predictive value. n=27.

Table 4: Proarrhythmic predictive values of electrophysiological parameters combined by multiplication

	RR	*PP*	*QT*	*QTc*	*LV MAPD*	*RV MAPD*	*ΔMAPD*	*STV_{RR}^{-1}*	*STV_{PP}*	*STV_{LV}*
RR	-	0.95	0.87	0.84	0.92	0.83	0.59	0.79	0.81	0.97
PP	0.89	-	0.89	0.88	0.94	0.95	0.64	0.79	0.75	0.99
QT	0.89	0.93	-	0.77	0.86	0.91	0.58	0.79	0.76	0.98
QTc	0.89	0.92	0.89	-	0.85	0.86	0.57	0.78	0.75	0.96
LV MAPD	0.83	0.91	0.89	0.86	-	0.88	0.59	0.81	0.77	0.96
RV MAPD	0.79	0.87	0.83	0.81	0.74	-	0.58	0.84	0.75	0.90
ΔMAPD	0.51	0.52	0.52	0.50	0.52	0.52	-	0.69	0.72	0.59
STV_{RR}^{-1}	0.68	0.66	0.66	0.64	0.68	0.64	0.55	-	0.59	0.83
STV_{PP}	0.71	0.70	0.71	0.70	0.71	0.67	0.70	0.64	-	0.81
STV_{LV}	1.00	1.00	1.00	1.00	1.00	1.00	0.81	0.95	0.90	-

Area under the receiver-operating characteristic plots for electrophysiological parameters combined by multiplication. Since low STV_{RR} suggests increased risk of TdP (Table 3), the multiplication algorithm was altered to $A \times STV_{RR}^{-1}$. Values with grey background are derived from electrophysiological parameters after the administration of dofetilide. n=27.

Discussion

With the present study we confirm that baseline STV_{LV} is persistently increased in chronic-AVB dogs with remodelled hearts, when compared to dogs in acute AVB. The novel findings are that the increased STV_{LV} is a result of ventricular remodelling and independent of heart rate or STV_{RR}. Secondly, STV_{RR} is significantly increased immediately after AVB, which persists over weeks during cardiac remodelling, whereas STV_{PP} is only elevated secondary to the ventricular remodelling. This is the first study to document that I_{Kr} block causes comparable QTc prolongation across a range of cardiac pathologies from healthy to compensated hypertrophy, whereas STV_{LV} is transiently increased only in the individual that later develops TdP.

Cardiac ventricular remodelling and BVR

Volume-overload induced cardiac remodelling after AVB has been subject of intense interest over the years. Next to the structural and functional remodelling processes in dogs, a profound electrical remodelling takes place, contributing to the increased proarrhythmic phenotype present after 3 weeks of AVB. Table 1 show that the AV-nodal ablation causes bradycardia, whereas the repolarisation-dependent parameters are not altered at this acute stage, especially when corrected for heart rate. Earlier studies have shown that cardiac output is momentarily decreased at this point despite an adrenergically induced increase in stroke volume[16,17]. Over weeks, cardiac output is restored as biventricular eccentric hypertrophy develops to facilitate an increase in stroke volume and the adrenergic drive levels off[14,17,18]. At this point, electrical remodelling has prolonged the ventricular repolarisation partly through a downregulation of I_{Kr} and I_{Ks}, an upregulation of the sodium-calcium exchange current, and increased calcium release from the sarcoplasmic reticulum[11,19]. The results of the present study strongly suggest that increased BVR is another hallmark of ventricular electrical remodelling. Together, the remodelling processes render the dogs with chronic AVB susceptible to drug-induced and spontaneous TdP arrhythmias[9,10,13,14,16,20]. In Table 1, we confirm our earlier results that the repolarisation-dependent parameters like QT, QT_c, LV and RV MAPD alongside interventricular dispersion of repolarisation duration are all increased at chronic AVB. Figure 2A shows that BVR is independent of the acute bradycardia and only increases as a consequence of ventricular remodelling, like the other repolarisation parameters in Table 1. Interestingly, beat-to-beat variability of the ventricular rate (STV_{RR}) is significantly increased after AVB but does not alter further during remodelling to chronic AVB. Thus, the present study shows for the first time that the increased STV_{LV} seen at chronic AVB is a consequence of the remodelling processes rather than directly induced by a decreased ventricular rate, whereas an increased STV_{RR} is intrinsic of the idioventricular rate and not affected by cardiac remodelling (Fig. 2A). Furthermore, this implies that bradycardia does not contribute to BVR in non-remodelled circumstances.

Drug-induced TdP in chronic-AVB dogs

Administration of QT-prolonging drugs causes a significant increase in STV_{LV} prior to the occurrence of TdP (Fig. 5), as have been shown previously[5,20,21]. Furthermore, QT-prolonging agents free of proarrhythmia in the chronic-AVB dogs do not induce an increase of STV_{LV}[5,20]. The present study was not undertaken to demonstrate the proarrhythmic properties of dofetilide, which has been identified previously by ourselves and in large clinical trials[10,22]. Rather, we wished to investigate the relationship between various electrophysiological parameters, including BVR, and individual susceptibility to drug-induced TdP.

The theory of multiple hits on repolarisation suggests that several consecutive reductions of repolarisation reserve are required for the initiation of TdP[20,23]. In our setting, the first perturbation of repolarisation is the AV-nodal ablation causing bradycardia. Secondly, weeks of cardiac remodelling sets the stage for TdP. Finally, a combination of anaesthesia and fast intravenous administration of a proarrhythmic drug triggers a series of events, including increased BVR, prolonged and increased spatial heterogeneity of repolarisation duration, occurrence of early afterdepolarisations and extrasystoles, often culminating in reproducible TdP arrhythmias.

The difference between resistant and inducible chronic-AVB dogs (Table 2) despite identical hits on repolarisation reserve is likely to be dependent on the genetic background of the dogs, although further investigations into this area are needed before this remains clear. Table 2 shows that resistant dogs have a faster idioventricular rate than TdP-inducible dogs, raising the possibility that these animals experience less electrical remodelling. This could be secondary to different levels of contractile or structural remodelling in the two groups, however these remodelling aspects were not determined in the present study. Nevertheless, cardiac remodelling is essential for the induction of arrhythmia.

Beat-to-beat variability

By using Poincaré plots, STV is one way of analysing BVR. A dose-dependent TdP occurrence after *d*-sotalol was tightly associated with the transient increase in STV_{LV}, whereas the absence of TdP after a QT prolonging drug is reflected in an unchanged STV_{LV}[5]. Later, we documented that anti-torsadogenic preventive or interventional treatments are associated with stabilised or even decreased STV_{LV}[20].

In the sinus-rhythm dogs, dofetilide increased STV_{PP} and STV_{RR} but had no effect on either STV_{QT} or STV_{LV} (Figs. 2 and 3). Thus, the repolarisation in these dogs is strong enough to compensate for the irregular diastolic intervals and keeping action-potential duration constant. Furthermore, the overall range of RR changes within the 30 beats in a dog is <10% of the mean RR interval, probably too small to significantly influence action potential duration. Interestingly, the beat-to-beat heart-rate correction did not transfer the dofetilide-induced increase in STV_{RR} to differences in STV_{QTc}. Caution should be taken in interpreting these results, as heart-rate correction formulas have not been designed to work on an

immediate beat-to-beat basis. Furthermore, changes in repolarisation tend to lag behind the heart-rate change[24,25].

In an awake study using telemetry monitoring of 6 dogs in sinus rhythm, dofetilide (30 μg/kg i.v.) prolonged the QT interval by 15% without significant effect on the heart rate[26]. STV_{QT} calculated over 100 beats did not change significantly (6.5±3.7 versus 10.4±3.9 ms; *P*=NS) as a consequence of dofetilide administration. Thus, compared to the present study (Table 1), the awake situation shows a shorter QT at baseline and a smaller dofetilide-induced increase in the QT interval, however a larger baseline STV_{QT}. Unfortunately, STV_{RR} was not quantified in the awake study, as a considerable respiration-induced RR-interval variability in conscious dogs is known to exist[27], which could contribute to a higher STV_{QT} at baseline. We and others have shown that this dose of dofetilide does not cause TdP arrhythmia in sinus rhythm dogs either with or without anaesthesia[10,26].

Beat-to-beat QT interval measurements in dogs with AVB are hampered by P waves coinciding with the end of the T wave (Fig. 1). When such beats are skipped in the analysis, the direct beat-to-beat consecutiveness is lost and sensitivity declines[5]. Furthermore, we have previously shown that STV_{RV} is a poor predictor of drug-induced TdP[5,13]. In the anaesthetised dogs with AVB, STV_{LV} is thus the preferable measure of repolarisation lability.

The shortened PP interval directly after AVB (Table 1) has been shown earlier[14,17,18] and is generally attributed to the increased adrenergic activity compensating the acute drop in cardiac output. The PP intervals are normalized to pre-AVB levels after 2 weeks of AVB[14,17,18]. Still, long PP intervals seem to be a proarrhythmic risk factor in the chronic-AVB dogs (Table 2), which could indicate that either low adrenergic or high vagal tone contributes to the proarrhythmic trigger. A high adrenergic tone, suggested by a short PP interval, would theoretically imply a β-adrenoceptor-mediated activation of the partly down-regulated I_{Ks}, preventing an excessively prolonged and unstable action potential, thereby possibly serving as a safety factor, reducing TdP inducibility. This is probably a delicate balance of the autonomic nervous system, as intense and acute β-adrenergic stimulation is proarrhythmic[28].

Interestingly, the wide range of STV_{PP} values in the dogs with chronic AVB (Fig. 4) indicates different levels of atrial remodelling despite comparable ventricular remodelling after AVB. This could possibly be based on atrial vulnerability to stretch as the atria are regularly contracting against closed valves after complete AVB. The chronic-AVB dogs with the largest STV_{PP} are thus sensitive to atrial stretch induced by ventricular contraction. Further research is needed to quantify and understand this atrial remodelling in detail.

Electrophysiological parameters predicting TdP

Figure 6 illustrates receiver-operating characteristics (ROC) for RR, QT, and STV_{LV} with and without pharmacological challenge showing the specificity as a function of sensitivity over a large range of cut-off points to avoid the bias of choosing artificial cut-off points. The area under the curve indicates the predictive value of the given parameter, where an area of 1 indicates 100% specificity and 100% sensitivity for at least 1 cut-off point. At baseline, the most powerful predictors of individual susceptibility to TdP are long PP intervals and elevated STV_{LV} (Table 3). By combining 2 electrophysiological parameters through simple

multiplication, the predictive power could be increased in many instances (Table 4). At baseline, multiplication of the PP interval and STV_{LV} gives a rather arbitrary value but a high TdP-predictive power. Dofetilide caused a significant increase in STV_{LV} in the TdP-inducible dogs only (Table 2), but more importantly all values of STV_{LV} increased to over 3.0 ms in the group, whereas none of the resistant dogs reached this level. Hence, there is a total separation of the 2 groups by means of STV_{LV} (Fig. 6) and the resulting area under the ROC curve is 1 (Table 3). This finding is further accentuated by the presence of persistently elevated BVR above 3.0 ms in retrospectively analysed chronic AVB dogs that died suddenly under conscious, drug-free circumstances[13].

In the present study, ΔMAPD represented spatial dispersion of repolarisation duration, whereas temporal dispersion was characterised by BVR. Interventricular dispersion of MAPD may not directly represent the spatial dispersion required for the perpetuation of triggered TdP[29]. Nevertheless, increased ΔMAPD is likely to suggest steeper gradients of spatial repolarisation dispersion that could infringe an early-afterdepolarisation-triggered extrasystole. Generally, interventricular dispersion is larger that intraventricular dispersion of repolarisation duration[30], however the latter was not assessed in the present study. Discrete ventricular areas with substantial discordant BVR could give rise to moments of significant intraventricular dispersion setting the stage for TdP arrhythmias. Administration of dofetilide to chronic AVB dogs (Table 3) increases the proarrhythmic substrate (e.g. QT interval and spatial dispersion of repolarisation), whereas triggers (e.g. elevated BVR and extra systoles) are only present in the inducible dogs.

Limitations

This study limits BVR measurements to anaesthetised dogs, suggesting precaution when extrapolating TdP-predictive values to the clinical setting. Presently, there is no satisfactory cellular explanation for the mechanism underlying BVR. As this was not a serial investigation, the electrophysiological characteristics of a dofetilide-challenge in a chronic-AVB dog cannot be tracked back to the sinus-rhythm situation. STV_{QT} after AVB could not be determined as precise measurements occasionally are hampered by P waves (Fig. 1).

Clinical Implications

Identification of the patient susceptible to drug-induced life threatening TdP arrhythmias is difficult, and the list of available drugs prolonging cardiac repolarisation is increasing, especially among non-cardiovascular drugs[31]. In-hospital initiation of therapy is standard for an increasing number of drugs to minimise the risk of TdP-induced cardiac mortality. Novel proarrhythmic parameters may enhance the quality and reduce the costs of the pre-treatment evaluation of the individual patient receiving potentially torsadogenic medication.

Pharmacological pre-screening of patients is not ideal[4], however in the present study, dofetilide was used as a tool to elicit proarrhythmic individuals. Our study suggests that baseline BVR, possibly in combination with other electrophysiological parameters, may contribute to the individual risk stratification of patients. Preliminary results suggest that this may be feasible also in the clinical setting[32].

Conclusions

Beat-to-beat variability of repolarisation duration quantified by STV_{LV} is persistently increased by ventricular remodelling but not directly by the slower ventricular rate after AVB. Neither RR intervals nor variability of the RR interval influence BVR. Only hearts prone to TdP express a transient increase in BVR upon dofetilide challenge. Thus, BVR may aid the identification of the TdP-susceptible patient before manifest proarrhythmia.

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Chapter 4

Atrial-specific drug AVE0118 is free of torsades de pointes in anesthetized dogs with chronic complete atrioventricular block

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Heart Rhythm, Vol 3, No 11, November 2006

Abstract

Background: The novel compound AVE0118 has been shown to prevent and terminate persistent atrial fibrillation. AVE0118 blocks I_{Kur}, I_{KAch}, and I_{to} leading to prolongation of atrial repolarization with no increase in ventricular repolarization duration. This suggests that AVE0118 is devoid of proarrhythmic side effects. Experimentally, AVE0118 was antiarrhythmic against some specific ventricular arrhythmias. We investigated the pro- and antiarrhythmic effects of AVE0118 in anesthetized dogs with chronic complete atrioventricular block (CAVB), known for a high proclivity for torsades de pointes (TdP)

Methods: AVE0118 was administered i.v. as fast (0.5 mg/kg/5 min) and slow infusion (3 or 10 mg/kg/60 min). Dofetilide was given to induce TdP. ECG and monophasic action potentials (MAP) were recorded. Short-term beat-to-beat variability (STV) of the left ventricular (LV) MAP duration (MAPD) was calculated. We examined whether AVE0118: 1) causes ventricular proarrhythmia (both infusions), 2) prevents drug-induced TdP (slow infusion + dofetilide after 30 min), 3) abolishes dofetilide-induced TdP (fast infusion).

Results: 1) At 0.55±0.10 µg/mL (fast infusion at 10 min), AVE0118 did not increase ventricular repolarization nor did it induce TdP while the right atrium $MAPD_{50}$ and $MAPD_{90}$ were significantly increased by 26±9 and 10±5 %, respectively ($P<0.05$ vs. baseline). 2) At 1.9±0.5 µg/mL and 6.1±1.2 µg/mL (30 min of 3 or 10 mg/kg/h), AVE0118 did not induce TdP (0/6 and 0/4) nor did it prevent dofetilide-induced TdP (6/6 and 2/2). Dofetilide significantly increased all repolarization parameters, including STV: from 2.1±0.4 to 4.6±1.8 ms ($p<0.05$ vs. baseline), which were not changed by AVE0118 (to 2.1±0.3 ms after 30 min). 3) Rapid infusion of AVE0118 did not suppress dofetilide-induced TdP.

Conclusions: In the anesthetized CAVB dog, the atrial-specific drug AVE0118 is 1) free of TdP and 2) has no antiarrhythmic properties against dofetilide-induced TdP 3) AVE0118 does not influence STV.

Introduction

Atrial fibrillation is the most prevalent arrhythmia in the Western world and a major determinant for morbidity and mortality by its complications[1-3]. Clinically available antiarrhythmic drugs are used to control the ventricular rate, to prevent recurrence or to terminate atrial fibrillation (class-III antiarrhythmics). The benefit of the latter drugs is limited because their ventricular action (prolonged QT interval) is often associated with an increased risk for torsades de pointes arrhythmias (TdP).

In the search of safe drugs to terminate atrial fibrillation[4], the pharmacological industry has focused on "atrial-specific" drugs[5,6]. One of these drugs is AVE0118 (Sanofi-Aventis), which in addition to blocking the ultrarapid delayed-rectifier potassium current I_{Kur}, has been shown to block the acetylcholine-dependent potassium channel I_{KAch} and the transient outward current I_{to}, without affecting the delayed-rectifier currents I_{Kr} and I_{Ks} or the inward rectifier current I_{K1}[7]. Also most of the currents are present throughout the myocardium, I_{Kur} and I_{KAch} have been shown more abundantly in atrial than ventricular myocytes[8-12].

It has been shown in animal models that AVE0118 prolongs the atrial refractoriness and repolarization in a dose-dependent manner without affecting the QT or QTc time[13,14]. Moreover, AVE0118 prevented the induction of atrial fibrillation in normal pigs[13] and in remodeled atria of the goat and converted persistent atrial fibrillation in this goat model[14]. The absence of effects on ventricular repolarization parameters suggests that AVE0118 is devoid of ventricular proarrhythmic side-effects. In the present study, we sought evidence to confirm the lack of ventricular proarrhythmia in a highly susceptible animal model for drug-induced TdP: the anesthetized dog with chronic complete atrioventricular block (CAVB)[15,16].

Other investigators have pointed to the possibility that the I_{to}-blocking properties of AVE0118 could have ventricular antiarrhythmic effects: AVE0118 prevented a) ventricular tachycardia/fibrillation (VT/VF) in an experimental model of Brugada syndrome[17] and b) VT/VF induced by myocardial ischemia and exercise[18]. Thus, our second aim was to evaluate whether AVE0118 also has ventricular antiarrhythmic (preventive and suppressive) properties against repolarization dependent arrhythmia: dofetilide-induced TdP.

Methods

All experiments were 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" and under the regulations of "The Committee for Experiments on Animals" of the University of Utrecht and Maastricht University, The Netherlands.

General Protocol

Twelve adult dogs were included for these serial experiments, performed under anesthesia. After overnight fasting, the dogs (23$\pm$4 kg body weight, 7 female and 5 male) were sedated with 0.5 mg/kg methadone, 0.5 mg/kg acepromazine and 0.5 mg atropine i.m. Anesthesia

was induced with sodium pentobarbital (25 mg/kg i.v.) and maintained by halothane (0.5-1%). The dogs were artificially ventilated. Proper care was taken before, during and after the experiments including a thermal mattress to maintain body temperature, fluid administration to prevent volume depletion (0.5 L 0.9% NaCl), administration of antibiotics and analgesics (buprenorphine 0.015 mg/kg i.m.).

CAVB was induced by radiofrequency ablation. A 7-French steerable catheter, with a 4-mm tip (RF Marinr™ , Medtronic CardioRhythm, San Jose, CA, USA) was positioned across the tricuspid valve to record a large atrial and a small His-bundle potential. Temperature-controlled radiofrequency energy, with a power limit of 35-50 W and a target temperature of 70 ºC was delivered from a 500-Hz generator (Atakr™ , Medtronic CardioRhythm, San Jose, CA, USA) for maximal 2 min, between the thermocouple electrode of the ablation catheter and an adhesive pad applied on the back of the dog[19].

Experimental Protocols

After 4±1 weeks of chronic AV block the dogs were subjected to two different infusion schemes with AVE0118 (fast and slow) in order to determine its ventricular proarrhythmic and antiarrhythmic effects. The fast i.v. infusion of 5 min was chosen for two reasons: a) it is identical to the infusion time we have used in the past for other drugs tested for proarrhythmic properties[16,20] and b) the fast administration allows testing of antiarrhythmic suppressive properties against dofetilide-induced TdP. The slow infusion (60 min) was chosen to mimic the clinical administration scheme to reach sufficiently high plasma levels of AVE0118. Further more we could also determine the preventive antiarrhythmic effects of AVE0118 against dofetilide-induced TdP. Dofetilide has been studied extensively over the recent years[16,19,20].

Part A: fast infusion: 0.5mg/kg/5 min

1: first experiment: atrial and ventricular electrophysiological and proarrhythmic effects of AVE0118 (n=5),
2: second experiment: antiarrhythmic, suppressive effects of AVE0118 against dofetilide-induced TdP (n=3).

The average time between two experiments in a dog was 2±1 weeks.

Part B: slow infusion: 3mg/kg/h

3A: first part of the experiment: ventricular electrophysiological and proarrhythmic effects of AVE0118 (n=6),
3B: followed 30 min later by dofetilide to study the preventive antiarrhythmic effects of AVE0118.

Part C: slow infusion: 10 mg/kg/h

4A: first part of the experiment: ventricular effects of AVE0118 (n=5).
4B: preventive potential against dofetilide-induced TdP (n=2) at 30 min.

Standard 6-leads ECGs with precordial registrations were combined with endocardial monophasic action potential (MAP) recordings in all experiments and continuously stored. MAPs (EP Technologies, Sunnyvale, CA) were gathered from left and right ventricular (LV and RV) endocardial sites. In the fast infusion experiments, we also recorded a right atrial (RA) endocardial MAP. Before drug administration, programmed electrical stimulation was performed to ascertain that no arrhythmic activity could be induced in these CAVB dogs at baseline. The stimulation protocol delivered through the RV-MAP catheter used a pulse width of 2 ms and amplitude of twice the threshold for capture. The pacing protocol consisted of two different pacing modes: a basic train of 8 stimuli followed by an extrastimulus (the interval of the extrastimulus was shortened from 500 ms in steps of 50 ms till 350 ms) or a short-long-short sequence (1x400ms with a 800 ms pause and an extrastimulus or 4x600 with 1200 ms pause and extra from 500 to 350 ms with steps of 50 ms)[21]. The pacing protocol did not induce TdP and therefore no dogs were excluded.

Drugs and Plasma Levels

AVE0118 was provided by Sanofi-Aventis Germany GmbH, Frankfurt, Germany in a solution of 5 mg/mL. Dofetilide, 1 mg was dissolved in 100 µL HCl (0.1mol/L) and then diluted in 0.9% NaCl to a concentration of 0.05 mg/mL and administered in a dose of 0.025 mg/kg/5 min. Dofetilide infusion was stopped when TdP occurred.

During the experiment, blood samples were taken at 0, 5, 10, 15, 20, 30, 40, 50, 60 min to determine AVE0118 plasma levels. Plasma was stored at -18 ºC and analyzed by Sanofi-Aventis Germany GmbH, Frankfurt, Germany.

Data Analysis

RR and QT interval in lead II, LV and RV MAP duration (LV and RV MAPD) at 90% repolarization and RA MAP duration (RA MAPD) at 50 % and 90 % repolarization were measured off-line using a custom-made computer program (ECGView). Data were averaged from 5 consecutive beats. At baseline, two measurements were performed (t = -5 min, and t = 0 min) and after AVE0118 the parameters were determined every 10 min. QT intervals were corrected for heart rate (QTc) with the formula of Van de Water et al[22]. The interventricular dispersion of repolarization (Δ MAPD) was calculated as the difference between the LV and RV MAPD. Beat-to-beat variability of repolarization duration was quantified with short-term variability (STV) from LV MAPD of 30 consecutive beats:
$STV_{LV} = \sum(\text{LV MAPD}_{n} - \text{LV MAPD}_{n-1}) / 30 * \sqrt{2}$[23].

Arrhythmic Outcome

TdP was defined as a polymorphic ventricular tachyarrhythmia with a twisting shape of at least 5 consecutive beats. A dog was defined inducible when TdP occurred at least three times. When TdP did not stop spontaneously in 10-15 seconds or when arrhythmia deteriorated into VF, electrical cardioversion was performed through the thoracic patches placed in advance. If TdP recurred during of a period longer than 10 min, levcromakalim (10 µg/kg i.v.), an $I_{K,ATP}$ opener, was used to restore a regular rhythm.

Statistics

Pooled data are expressed as mean ± standard deviation in ms. Comparisons were made using repeated-measures ANOVA with a post-hoc Bonferroni test. This apply also for the data shown in the tables in which specific time points were chosen to be shown. P values <0.05 were considered significant.

Results

Electrophysiological effects with the fast infusion 0.5 mg/kg/5min AVE0118

Fast administration of AVE0118 resulted in maximal plasma levels (0.55±0.10 µg/mL) at 10 min (Figure 1, upper panel). Duration of the RA MAP increased accordingly, reaching maximal values at 10 min (Figure 1, lower panel), with a decline in time thereafter. A representative individual example is given before (control) and after AVE0118 is shown in Figure 2. At the different measuring points after AVE0118, we noticed a change in the morphology of the RA MAP: there was a greater effect on $MAPD_{50}$ than $MAPD_{90}$. This hold true both for the absolute increase (with 27±7 ms at $MAPD_{50}$ vs. 15±7 ms at $MAPD_{90}$, $P<0.05$) as well as for the relative increase (26±9 % versus 10±5 %, respectively, Table 1).

At the ventricular level, there was no detectable change in any of the repolarization parameters: $QT_{(C)}$ time, LV and RV $MAPD_{90}$ (Figure 2 and Table 1). Finally, the fast infusion of AVE0118 was free of any ventricular proarrhythmic activity in dogs that were susceptible for TdP after dofetilide.

Electrophysiological effects with slow infusion, 3 mg/kg/60min AVE0118

Compared to the fast infusion, the slower administration mode resulted in a more gradual increase in the plasma concentration of AVE0118. Maximal levels of AVE0118 reached higher levels (e.g. 1.9±0.5 µg/mL at 30 min, $p<0.05$ vs. fast) and were much more stable in time due to the continuous infusion (Figure 3, upper panel). Again AVE0118 did not change the ventricular parameters (Figure 3, lower panel), which now also included spatial (ΔMAPD) and temporal (STV_{LV}) dispersion (Table 2). An example is given in the left part of Figure 4. No arrhythmogenic activity was seen after this infusion of AVE0118.

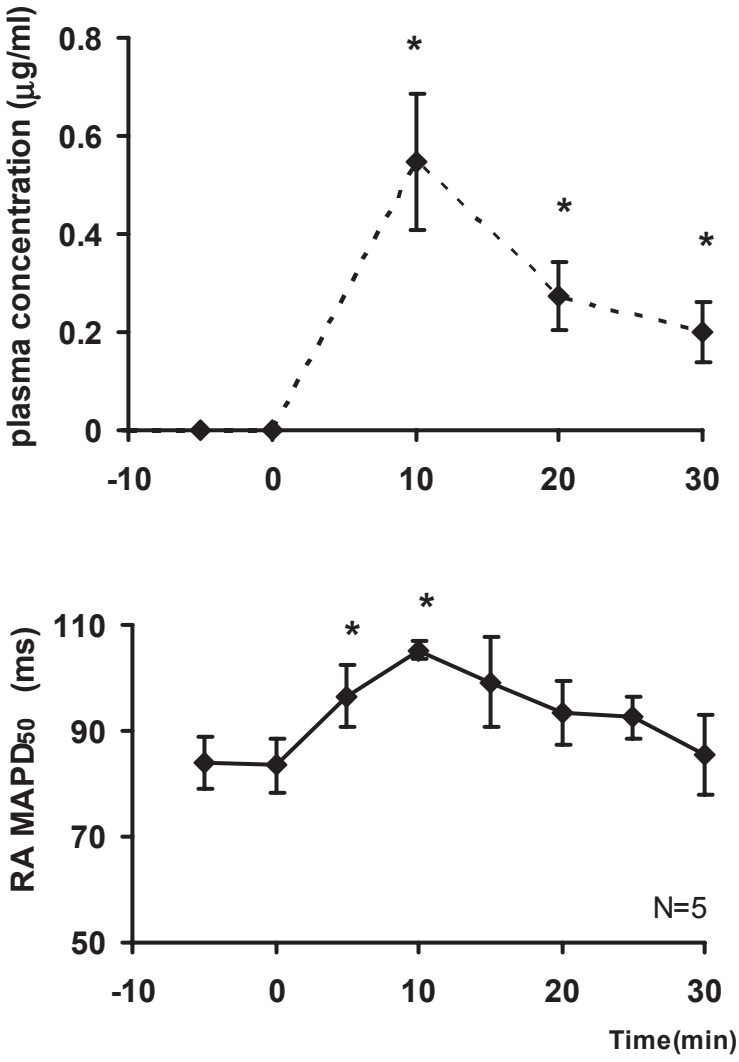

Figure 1. Electrophysiological effects of fast infusion AVE0118 with corresponding plasma concentrations.
The time dependent changes in plasma concentration (upper panel) and the duration of the right atrial monophasic action potential at 50% repolarization (RA $MAPD_{50}$, lower panel) after AVE0118 infusion are depicted. At t=0, AVE 0118 is given at a fast infusion lasting 5 min. In the lower panel, a temporary increase in RA $MAPD_{50}$ that reaches a maximum 10 min after the start of the infusion is seen. This increase follows the changes seen in plasma concentration in the upper panel. * = $p<0.05$ versus t=0 (baseline).

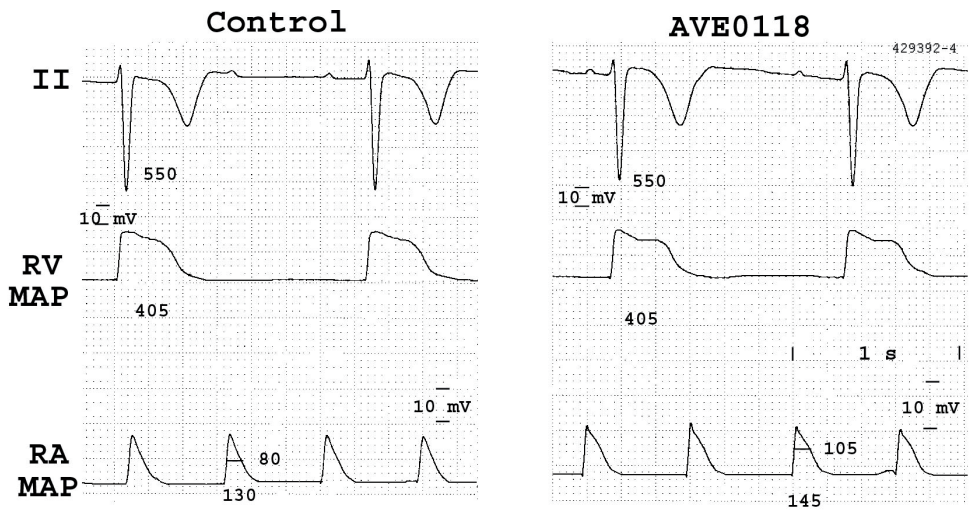

Figure 2. Atrial-specific effects of fast infusion AVE0118 in anesthetized CAVB dogs.
ECG lead II and two recordings of endocardially placed monophasic action potentials
in the right ventricle (RV MAPD) and atrium (RA MAPD) are depicted at 25 mm/sec before (left) and after fast infusion of AVE0118 (right panel). AVE0118 administration results in an increase in RA MAPD but does not change the ventricular parameters RV MAPD and QT time.

Electrophysiological effects with the slow infusion, 10 mg/kg/60min AVE0118

With this infusion the plasma concentration of AVE0118 reached a level of 6.1±1.2 µg/mL at 30 min (which is dose dependent, $p<0.05$ vs. 1.9±0.5 µg/mL at 3 mg/kg/60 min at 30 min). TdP was never induced with this dose and the ventricular electrophysiological parameters were not changed. However the emergence of a bidirectional VT in one dog prompted us to prematurely stop the infusion. Unexpectedly 2 dogs died in their cages in the first 24 hours after the experiment in which they received this infusion scheme (10 mg/kg/60 min) followed by dofetilide (after 30 min). Therefore we decided not to perform the dofetilide challenge in the other two experiments. Afterwards there was one more unexplained death in the first 24 hours following the operation resulting in a total mortality of 3/5 dogs to which 10 mg/kg/60 min was administered.

Antiarrhythmic effects of AVE0118 against dofetilide-induced TdP

Suppression

In three dogs, AVE0118 was given as a fast infusion in an attempt to suppress the dofetilide-induced TdP arrhythmias. There was no suppressive effect of AVE0118.

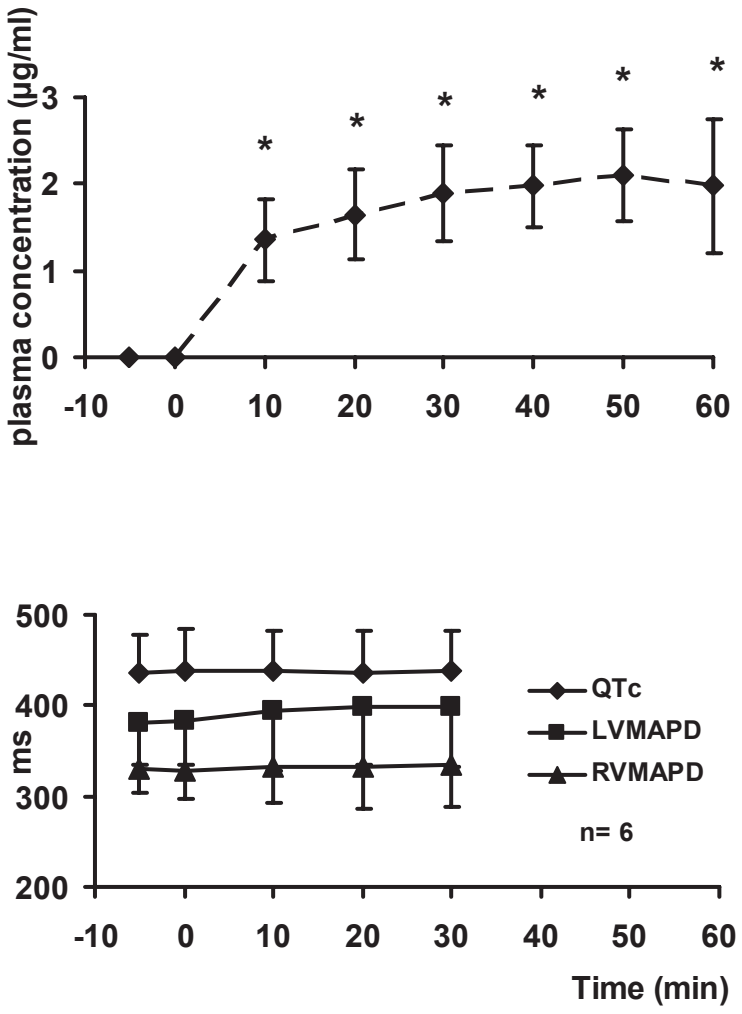

Figure 3. Electrophysiological effects of slow infusion AVE0118 with corresponding plasma concentrations.
The time-dependent changes in plasma concentration (upper panel) and in ventricular parameters representing repolarization (QTc time, LV and RV MAPD, lower panel) after AVE0118 infusion are depicted. At t=0, AVE 0118 is given as a slow infusion lasting 60 min. There is an increase in the plasma concentration that is at a steady state at 30 min when dofetilide has been co-administered. In the lower panel, evidence is provided that AVE0118 does not affect ventricular repolarization: there is no change in QTc, LV or RV MAPD. * = $p<0.05$ versus t=0 (baseline).

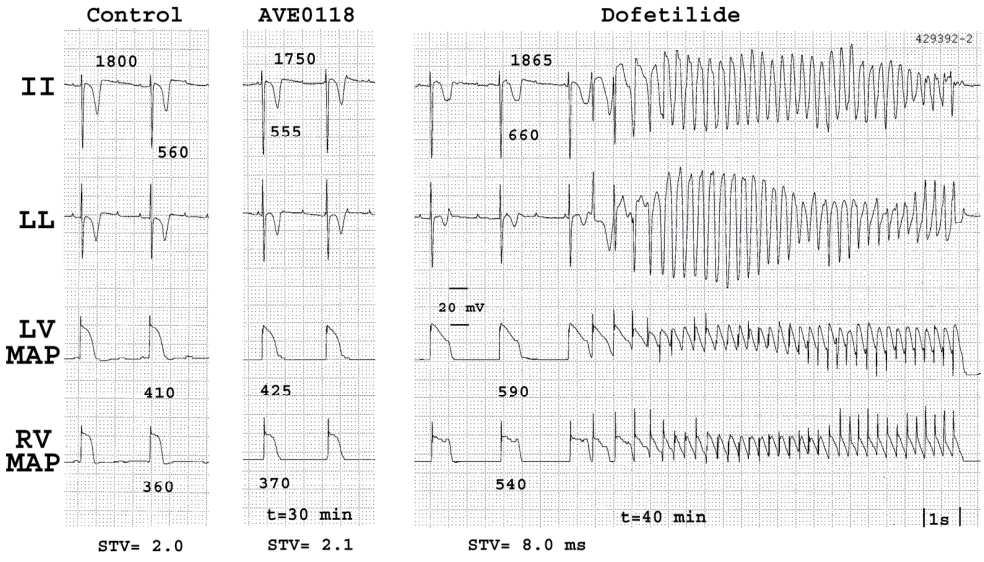

Figure 4. Proarrhythmic effects with AVE0118 and dofetilide.
Two ECG leads (II and a precordial lead LL), and two recordings of the endocardially placed monophasic action potentials in the left and right ventricle (LV and RV MAPD) are depicted at 10 mm/sec at baseline (left), after 30 min AVE0118 (middle) and in combination with dofetilide (right panel). Administration of AVE0118 did not change any ventricular parameter of repolarization, including beat-to-beat variability quantified as STV of the LV MAPD. After the addition of dofetilide, ventricular repolarization and STV is increased which precedes the occurrence of a TdP.

Prevention
The addition of dofetilide in the second and third series of experiments at 30 min AVE0118 (slow infusion 3 and 10 mg/kg/h) evoked TdP in all dogs tested (6/6 and 2/2). Dofetilide led to clear increases in the different repolarization parameters, including STV_{LV} (Table 2 and Figure 4). This increase of the repolarization parameters was not different from the increase seen after dofetilide alone (first series) or from that we observed in numerous other experiments with this drug. An example of a dofetilide-induced TdP is given in the right part of Figure 4.

Discussion

In the anesthetized CAVB dog, AVE0118 only prolongs repolarization in the atrium with no detectable pro- or antiarrhythmic effects in the ventricles. AVE0118 did not provoke or suppress TdP.

Table 1: The effects of the fast infusion (AVE0118, 0.5 mg/kg/5 min) on the electrophysiological parameters.

	Baseline	10 min AVE0118	Change(%)
RR (ms)	1377±137	1367±136	- 0.5±0.5
RA $MAPD_{50}$ (ms)	83±5	105±2 *	+ 26±9
RA $MAPD_{90}$ (ms)	153±2	169±9 *	+ 10±5
QT (ms)	501±33	507±33	+ 1±1
LV $MAPD_{90}$ (ms)	426±40	433±32	+ 2±2
RV $MAPD_{90}$(ms)	361±35	364±36	+ 1±1

*, P<0.05 vs. baseline

Table 2: The effects of the slow infusion (AVE0118, 3 mg/kg/60 min) on electrophysiological parameters:

	Baseline	30 min AVE0118	+ Dofetilide
RR (ms)	1260±340	1215±348	1251±383
QT (ms)	460±64	457±62	522±95 *
QTc (ms)	438±47	438±44	500±74 *
LV $MAPD_{90}$ (ms)	384±49	398±64	481±103 *
RV $MAPD_{90}$ (ms)	329±32	334±45	378±92
ΔMAPD (ms)	49±31	64±26	103±70
STV LV MAPD (ms)	2.1±0.4	2.1±0.3	4.6±1.8 *
TdP (nr. of dogs)	0 of 6	0 of 6	6 of 6

*, P< 0.05 vs. baseline

Atrial-specific drugs

In recent years, the pharmaceutical industry has developed a number of compounds for the treatment of atrial fibrillation. These drugs (e.g. AVE0118, RSD1235, AZD7009) are classified as "atrial-specific drugs", because electrophysiologically their dominant action seems to be restricted to the atria. These drugs are now in phase II or III of clinical development[24,25] and they have in common that they block the potassium current I_{Kur}[5]. However, their action is not restricted to this (atrial-specific) ion current. For instance, it has been shown that AVE0118 also blocks I_{to} and I_{KACh}. The former is responsible for the notch (phase 1 of the AP) in many cardiac cells and blocking this ion channel may be part of the working mechanism of AVE0118.

In vitro, AVE0118 has been shown to increase the duration of the atrial action potential in different species (pig, rat, guinea pig, and rabbit) whereas it did not affect ventricular repolarization in guinea-pig papillary muscle[26].

Using atrial MAPs, we confirmed that i.v. administration of AVE0118 resulted in an increase in the RA MAPD, whereas the drug did neither cause any changes in the QT time nor in the ventricular MAPDs. For the latter, we have used two dosing schemes that resulted in the plasma concentrations that are known to have important atrial antiarrhythmic effects (discussed later).

It has been reported that AVE0118 changed the morphology of the atrial MAP, creating a more dominant prolongation of the plateau phase[13]. This could be beneficial for optimizing contractile performance (positive inotropic effect), as has been suggested, based upon results in intact atria of the fibrillating goat[27] as well as in cellular studies with AVE0118 on human tissue[28].

The more pronounced increase at the level of the plateau appears also to be present in the volume-overloaded atria of the CAVB dog. However this finding should be viewed with caution because 1) the RA MAP is difficult to record for longer periods as atrial MAPs can loose their amplitude making assessment of different MAP durations difficult; 2) the duration of the atrial MAP is smaller than in the ventricle, increasing the possibility that small measurement errors can be made.

Antiarrhythmic against atrial fibrillation

In the goat model of atrial fibrillation, AVE0118 was tested in order to prevent and/or to suppress (persistent) atrial fibrillation. At 3 mg/kg/h, there was a strong atrial electrophysiological effect in normal hearts that became even more pronounced when the atria were electrically remodeled (48 hrs of AF). This dose of AVE0118 prevented the AF induction by 68%, when dofetilide (20 µg/kg/5min) had no effect. In addition, up to 10 mg/kg/h AVE0118 resulted in dose dependent termination of (persistent) AF in up to 5/8 goats[14]. These antiarrhythmic effects were achieved by prolonging the atrial repolarization or refractoriness selectively with no effect on atrial conduction nor on ventricular repolarization (QT time). Similar observations were obtained in anesthetized pigs with unremodeled hearts[13].

Ventricular characterization now also included the determination of the duration of a local repolarization parameter: RV epicardial MAPD[13]. Other "atrial-specific compounds" have also been tested in a variety of arrhythmogenic models: e.g. AZD7009, NIP-142[29,30].

No TdP with AVE0118

On the basis of this atrial-specific action, no proarrhythmia in the form of TdP is anticipated. Because the QT time itself is not a suitable surrogate for drug-induced TdP[15,16,23], we have chosen to determine the proarrhythmic potential of AVE0118 in the anesthetized CAVB dog, a model with known high susceptibility of drug-induced TdP in this model [19, 31]. In this series of experiments, dofetilide caused TdP in all dogs tested (8/8 = 100%) whereas AVE0118 alone (all doses) did not cause TdP in the same dogs.

In addition to the actual recording of reproducible TdP, we also determined a number of ventricular electrical parameters. With dofetilide there was a general increase in these repolarization parameters (Table 2) including an abrupt increase in beat-to-beat variability of repolarization quantified as STV_{LV}. This parameter is now considered a biomarker for the prediction of drug-induced TdP. No change in STV_{LV} after AVE0118 is in line with the concept that repolarization is still under control and the risk for repolarization-dependent proarrhythmia is low or absent. Various drugs studied in the CAVB dog validate this paradigm. The absence of TdP after the infusion of drugs that prolong QT (amiodarone, moxifloxacin)[23,32] concurred with no change in STV of the LV MAPD, whereas actual recordings of TdP with drugs as the final hit were preceded by increases in STV_{LV}: d-sotalol, sertindole, NS-7, and dofetilide (see Table 2)[16,23,31]. Also interventions aimed to control STV_{LV} have been shown to be antiarrhythmic either in the prevention (hyperkalemia) or in the suppression (increased heart rate or levcromakalim) of drug induced TdP[33]. The cellular mechanisms behind beat-to-beat variability of repolarization are still under investigation, but presumably reflect alterations in intracellular Ca^{2+} handling[34].

AVE0118 is the first atrial-specific drug evaluated in CAVB-dog model. Whether other drugs have the same safe application needs to be addressed. The assessment of AZD7009 in other proarrhythmic models suggests that this is the case[35].

Is AVE0118 safe?

Drug-induced proarrhythmic events are not restricted to TdP. Any facilitation or provocation of any arrhythmic outcome by a drug must be judged before a drug can be considered safe. The lack of arrhythmia and the lack of effects on the measured parameters, including STV_{LV} in the remodeled heart of CAVB dog are reasons to conclude that AVE0118 dosing schemes of 0.5 mg/kg/5 min and 3 mg/kg/60 min are safe. However, at the higher dose (10 mg/kg/60 min) there is some concern given the occurrence of a bidirectional VT and an unexpected death in the first 24 hours following the operation in another experiment with this infusion scheme. Therefore judging the safety margin of AVE0118 at this high concentration is difficult.

We evaluated not only the proarrhythmic potential of AVE0118 for TDP but also its combination with dofetilide. This combination has been used to increase the antiarrhythmic efficacy of lower doses of AVE0118[36].

Lack of antiarrhythmic effects with AVE0118

AVE0118 blocks I_{Kur}, I_{KAch} and I_{to}. Because I_{Kur} and I_{KAch} are expressed more abundantly in atrial than ventricular myocytes, it is perceivable that block of I_{to} might be antiarrhythmic against specific ventricular arrhythmias. In a canine sudden cardiac death model administration of 1 mg/kg AVE0118 prevented VT/VF induced by ischemia and exercise, in seven of nine dogs[18]. In these experiments, there was no change in QTc duration to explain this strong antiarrhythmic effect.

In the RV-wedge preparation, VT/VF was induced by combined sodium and calcium channel blockade using terfenadine[17]. Phase 2 reentry was prevented in 5/5 wedges when they were pretreated with 7 μM AVE0118, effectively diminishing spatial dispersion of repolarization.

In the CAVB model with drug-induced repolarization-dependent arrhythmias, no antiarrhythmic effects were seen with AVE0118. This drug neither prevented nor did it suppress dofetilide-induced TdP. The drug also did not affect any of the electrical changes seen with dofetilide (increasing in duration and temporal dispersion of repolarization). It seems clear that the repolarization-dependent TdP arrhythmias are not helped by I_{to} blockade.

Conclusion

In the CAVB dog, AVE0118 prolongs atrial repolarization. In the ventricles, this agent is free of TdP, it does not affect the electrophysiological parameters including BVR nor does it have antiarrhythmic effects against dofetilide-induced TdP.

Acknowledgement

This study was in part financed by a grant from Sanofi-Aventis Germany GmbH, Frankfurt, Germany

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Chapter 5

Robust anti-arrhythmic efficacy of verapamil and flunarizine against dofetilide induced TdP arrhythmias is based upon a shared and a different mode of action

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Submitted

Abstract

Aims: The high predisposition Torsade de Pointes (TdP) in dogs with chronic AV-block (CAVB) is well documented. The anti-arrhythmic efficacy and mode of action of Ca^{2+} channel antagonists flunarizine (F) and verapamil (V) against TdP were investigated.

Methods: Mongrel dogs with CAVB were selected based on TdP inducibility with dofetilide (D). The effects of F and V were assessed after TdP and in different experiments to prevent D-TdP. ECG and ventricular monophasic action potentials (APs) were recorded. EP parameters and short-term variability of repolarization (STV) were determined. In *vitro*, F and V were added to determine their effect on 1) D induced early after depolarizations (EADs) in canine ventricular myocytes (VM), 2) diastolic Ca^{2+} sparks and 3) peak and late I_{Na}.

Results: D increased STV prior to TdP and in VM prior to EADs. Both drugs completely suppressed TdP and reversed STV to baseline values. Complete prevention of TdP was achieved with both drugs. Both prevented an increases in STV. EADs suppression was confirmed after F. Only F blocks late I_{Na}. Ca^{2+} sparks were reduced with V.

Conclusions: Robust anti-arrhythmic efficacy was seen with both Ca2+ channel antagonists. Their divergent electrophysiological actions may be related to different additional effects of the two drugs.

Introduction

In numerous pro-arrhythmic circumstances, $[Ca^{2+}]_i$-overload is the cause of Ca^{2+} leak through ryanodine receptor (RyR). Ensuing Ca^{2+} sparks increase cytosolic $[Ca^{2+}]_i$ which in turn may activate the sodium-calcium exchanger (NCX) to generate delayed afterdepolarizations (DADs) and triggered ventricular arrhythmias[1,2]. The "Ca^{2+}-antagonists" flunarizine (F) and verapamil (V) suppress DADs or DAD dependent ventricular tachycardias (VTs) either induced by ouabain[3-6] or by cathecholamines[6]. In addition, both drugs have also been shown to be effective against Torsade de Pointes (TdP) arrhythmias, whether seen in congenital[7] or in acquired long QT[8-13]. The latter, however, are more likely initiated by early afterdepolarizations (EAD) dependent triggered activity. The mechanisms underlying these EADs can be reactivation of $I_{Ca,L}$, a persistent I_{Na} or NCX mediated inward current[8,14-16]. Although both drugs belong to the category Ca^{2+} antagonists, they have additional actions[17,18], such as blocking the delayed rectifier current (I_{Kr}) that may negatively affect their anti-arrhythmic efficacy against repolarization dependent VTs. The canine model of chronic, complete atrio-ventricular block (CAVB) has been used to initiate both DAD and EAD dependent VTs[1,4,10,14,15]. Its enhanced susceptibility for triggered arrhythmias has been related to Ca^{2+} overload of the sarcoplasmic reticulum (SR) and to a diminished repolarization reserve[1,4,15,19]. In this setting, beat-to-beat variability of repolarization duration (BVR) has been shown to be a better parameter to predict proarrhythmic predisposition than QT-time[19,20].

The objectives of this study were to determine whether: 1) flunarizine and verapamil prevent and/or suppress dofetilide-induced TdP in dogs with CAVB, 2) these drugs improve repolarization reserve quantified by BVR, 3) flunarizine is effective against dofetilide induced increases in BVR and EADs in ventricular myocytes isolated from dogs with CAVB, and 4) their mode of action on Ca^{2+} sparks and late I_{Na} *in vitro* differ or not?

Methods

General

Animal handling was 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" and under the regulations of "The Committee for Experiments on Animals" of the Utrecht University, The Netherlands.

A total of 26 adult mongrel dogs (Marshall, USA; 23±3 kg, 16 females) were included. Four weeks after induction of complete AV-block, 22 animals underwent a dofetilide (0.025 mg/kg/5') test. In this group, 5 dogs were excluded because they had TdP at baseline (n=3) or they were non-inducible (n=2). Repeatability and reproducibility of arrhythmias have been well studied in this model[19].

In a second group of 4 animals with CAVB, verapamil and lidocaine were administered in combination to explore their effect on electrophysiological parameters.

All experiments were performed under complete anesthesia, induced with barbiturates and maintained during the experiments with isoflurane (1.5 %). The detailed description of experimental procedures, AV-node ablation, ECG and MAP recordings (with MAP duration, MAPD at 90 % repolarization), definitions and data analysis, including calculation of BVR, e.g. from the left ventricle (LV) MAPD as short term variability (STV_{LV}), were previously published[21,22]. Early ectopic activity was defined as ectopic beats (EB) initiating before the end of the preceding T wave. Distinction between single (SEB) and multiple ectopic beats (MEB) was made as the latter are considered more proarrhythmic[23].

Anti-arrhythmic protocols in vivo:

Experimental protocols were performed, separated by at least 2 weeks intervals:

1) Suppression protocol

Approximately 10 min. after the start of dofetilide when TdP was reproducibly seen, flunarizine (n=10) (2 mg/kg/2', Janssen Pharmaceutica N.V.) or verapamil (n=7) 0.4mg/kg/3' (Isoptin, Abbott) were administered to suppress pro-arrhythmic activity.

Validation that TdP remained present in the second 10 min period after dofetilide was recently provided[19]. To allow comparison of verapamil and flunarizine, the dose of verapamil chosen, had a similar negative inotropic effect in canines[24,25] as seen with the anti-arrhythmic dose of flunarizine. Moreover, these equipotent negative hemodynamic effects were confirmed in 6 sinus rhythm dogs using a 7F catheter (Sentron, Roden, Netherlands): LV end-systolic pressure decreased by 20% with both drugs: flunarizine from 94±9 to 75±9 mmHg and verapamil from 87±13 to 67±9 mmHg.

2) Prevention protocol

Whether vulnerability to dofetilide-induced TdP could be prevented by pre-treatment with flunarizine (n=8) or verapamil (n=6) was investigated in this set of experiments. The dose of dofetilide used was exact the same as in the previous experiment. Three animals were tested serially with both verapamil and flunarizine.

3) Combination of drugs

To test if the electrophysiological effects seen with flunarizine were due to a combined block of $I_{Ca,L}$ and Iate I_{Na}, verapamil (0.2mg/kg/1.5') was followed 5 min later by 1.5 mg/kg/1' lidocaine (Braun Melsungen AG, Germany), a preferential of late I_{Na} blocker[26].

In vitro experiments:

The following dosages of the drugs were used: 1 µM dofetilide, 1 µM and 10 µM flunarizine or verapamil.

a) Effects of flunarizine on cellular STV

Single myocytes from CAVB dogs were enzymatically isolated[27]. Action potentials were

triggered in whole-cell current clamp mode with 2 ms current injections at a cycle length of 2000 ms and recorded with PClamp9 software (Molecular Devices, Sunnyvale, Ca, USA). Action potential duration (APD) was measured at 90% repolarization and cellular STV was calculated from 30 successive APDs in the same way as from *in vivo* data[21,27]. Experiments were performed in Tyrode solution containing (in mmol/L): 137 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 11.8 HEPES and 10 glucose, pH 7.4. Pipettes had a resistance of 2-3 MΩ when filled with pipette solution, containing (in mmol/L): 130 KCl, 10 NaCl, 10 HEPES, 5 MgATP and 0.5 $MgCl_2$, pH 7.2. Similar to *in vivo* experiments, two experimental protocols were used:

Protocol 1: effects of flunarizine on baseline cellular APD and STV in 8 myocytes isolated from the left ventricle (LV) of 4 dogs.

Protocol 2: effects of flunarizine on dofetilide-induced EADs. If 1μM dofetilide induced EADs, flunarizine was added to the Tyrode solution to test its suppressive effect on EADs and dofetilide-increased APD and STV. For these experiments another 8 cells (n=4 RV and n=4 LV) from 5 dogs were used.

b) Effects of flunarizine and verapamil on INa

For recording peak and late I_{Na}, SCN5A-HEK 293 cells were superfused with bath solution containing (in mol/L): 140 NaCl, 4.0 KCl, 1.8 $CaCl_2$, 0.75 $MgCl_2$, and 5 HEPES (pH adjusted to 7.4 with NaOH). The pipette solution contained (in mmol/L): 20 CsCl, 120 CsF, 2 EGTA, and 5 HEPES (pH adjusted to 7.4 with CsOH). All experiments were performed at 21±1°C. Whole-cell membrane current was recorded as previously described[28]. Computer software (pCLAMP 10.0, Molecular Devices, Sunnyvale, CA) was used to generate voltage-clamp protocols. Patch-clamp amplifier (Multiclamp 700B, Molecular Devices) data were sampled at 5 kHz. Whole-cell capacitance was compensated using the internal voltage-clamp circuitry and about 75–80% of series resistance was compensated. Membrane potentials were not corrected for junction potentials that arise between the pipette and bath solution. Cells were held at –140 mV and dialyzed for 5 min before I_{Na} recording. Data analysis of all measured currents was performed using pCLAMP 10.0 and Origin 7.0 (MicroCal, Northampton, MA) software. To measure the extent of tonic block (first-pulse) by flunarizine or verapamil on peak I_{Na}, 24-ms depolarizing steps to –20 mV from a holding potential of –140 mV were applied to cells at a rate of 0.1 Hz. The magnitude of peak I_{Na} in the presence of drug was normalized to the respective control value. To measure the effect of flunarizine or verapamil on late I_{Na}, the normally small late I_{Na} was augmented by exposure of cells to 3 nM ATX-II, and the effect of drug to reduce the ATX-II-induced late I_{Na} was determined. Late I_{Na} was defined as the magnitude of I_{Na} between 200 and 220 ms after application of a 220-ms depolarizing step to –20 mV from a holding potential of –140 mV applied at a rate of 0.1 Hz.

c) Effects of flunarizine and verapamil on Ca2+ sparks

Abnormally high spontaneous Ca^{2+} release in diastole (Ca^{2+} sparks) were recorded in intact quiescent myocytes enzymatically isolated from a homozygous mice carrying the mutation R4496C of the cardiac ryanodine receptor ($RyR2^{R4496C+/+}$), which underlies catecholamine polymorphic ventricular tachycardia (CPVT)[29,30]. To measure the effects of verapamil and

flunarizine on spontaneous Ca^{2+} spark activity, cells were loaded with fluorescent Ca^{2+} indicator (Fluo-3 AM) as previously described[30]. Cells were recorded under continuous Tyrode perfusion before and following the addition of flunarizine or verapamil for 10 min. Tyrode solution contained (in mmol/L): 140 NaCl, 4 KCl, 1.1 $MgCl_2$, 10 HEPES, 10 glucose, 1.8 $CaCl_2$; pH=7.4, with NaOH).

Images were obtained by confocal microscopy (Meta Zeiss LSM 510, objective w.i. 63x, n.a. 1.2) in the line scan mode as previously explained. Image analyses were performed by homemade routines using IDL software (Research System Inc.). Images were corrected for the background fluorescence.

Statistical analysis

Pooled data are expressed as mean ± standard deviation except the result on I_{Na} where results are presented as mean ± sem. For the effects of drugs in time, comparisons were performed using a 1-way repeated-measures ANOVA followed by a Bonferroni correction. For non-parametric comparison Kruskal-Wallis test was used.

Results

1) Antiarrhythmic effects of flunarizine

a) Flunarizine suppression

Dofetilide induced TdP with a median duration of 6.8 sec, after 3.1±1 min. After adding flunarizine, all arrhythmias disappeared with the exception of some SEBs in one dog (figure 1 and table 1). These anti-arrhythmic effects remained present for more than 10 min. Thereafter, some MEBs returned, albeit less severe. Electrophysiologically, dofetilide increased all repolarization parameters (QT, QT_C, LVMAPD and RVMAPD) and STV_{LV} before the first ectopic beat (2.5±0.5 min. after start dofetilide). Flunarizine decreased the dofetilide-augmented STV_{LV} and all the other repolarization parameters, to a level similar to baseline (table 1 upper part and figure 1).

b) Flunarizine prevention:

Pretreating the same animals with flunarizine resulted in complete prevention of TdP (figure 2, upper part). During a 10 min. period, dofetilide could only induce few single EBs (6±10 bts/10 min). Flunarizine significantly decreased baseline STV_{LV} and QTc. After adding dofetilide, STV_{LV} remained at a level similar to baseline, whereas an increase in QTc could not be prevented by this drug completely (Figure 2 and Table 1, lower part).

c) Effect of flunarizine on baseline cellular BVR and on dofetilide-induced EADs

In untreated isolated myocytes from dogs with CAVB, flunarizine shortened (at 10 min) both APD (from 418±116 ms in baseline to 312±74 ms, $p<0.05$) and cellular STV_{APD} (baseline 20±10 ms to 11±4 ms, $p<0.05$). The time course of changes in APD and STV during an experiment is shown in figure 3A.

Table 1

	Baseline 1	Dofetilide	Flunarizine
RR	1181±87	1291±140	1219±251
QT	436±44	566 ±29 *	435±36 $
QT_c	421±49	553±40 *	425±38 $
LV MAPD	355±35	492±53 *	367±42 $
RV MAPD	310±32	395±68 *	333±30 $
∆MAPD	51±28	97±56 *	48±32 $
STV_{LV}	1.8±0.5	4.5±1.5 *	1.5±0.6 $
TdP	0±0	11±8 *	0±0 $
MEB	0±0	13±14 *	0±0 $
SEB	1±2	48±58 *	1±3 $
	Baseline 2	**Flunarizine**	**Dofetilide**
RR	1239±329	1291±390	1410±462
QT	422±51	380±50 *	494±92*#
QT_c	413±51	369±41 *	476±77 *#
LV MAPD	299±44	277±36	380±65 *#
RV MAPD	286±39	275±44	348±73 *#
∆MAPD	42±27	22±16	38±42
STV_{LV}	1.5±0.6	1.0±0.5 *	1.4±0.5
TdP	0±0	0±0	0±0
MEB	0±1	0±0	0±0
SEB	3±6	3±5	6±10

Maximal effects of flunarizine (5 min) in suppression experiments (upper part) and at the end of the infusion (2 min) in prevention experiments are shown (lower part). Arrhythmias are quantified as average number of events per 10 minutes, except for the pretreatment with flunarizine (lower part) where after 5 min dofetilide was added. All EP parameters are expressed in ms and arrhythmias as average number per time interval. *, p<0.05 vs. baseline; $, p<0.05 vs. dofetilide; #, p<0.05 vs flunarizine pretreatment.

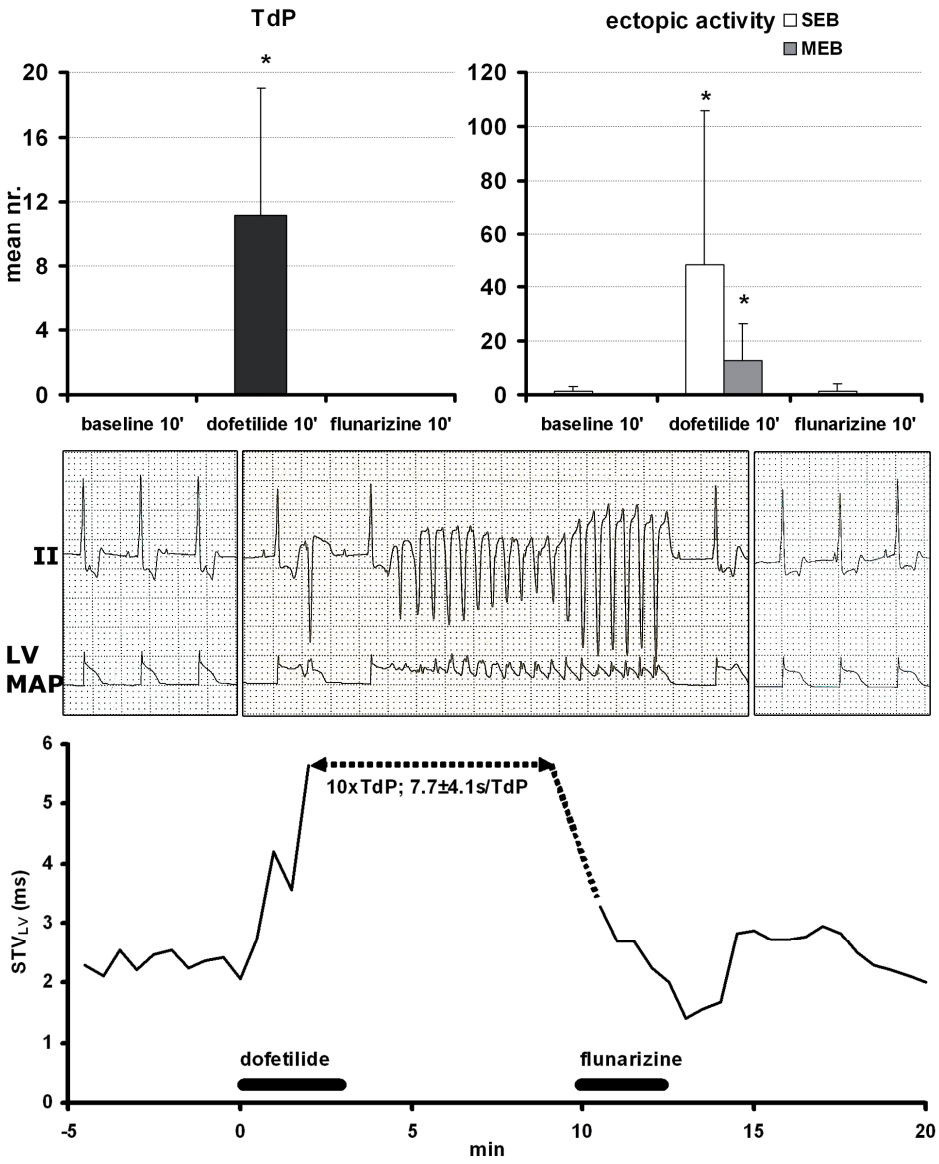

Figure 1. Upper panel: anti-arrhythmic effects of flunarizine (suppression) against dofetilide-induced TdP (left) and ectopic activity (right) is shown with an individual example (middle part) of lead II ECG and LV MAP recordings (printed at 10 mm/s speed and calibrated at 1 mV per cm for ECG and 20 mV for the MAP recording) on scale paper in baseline (left), with TdP (middle) and after flunarizine. Lower panel illustrates continuous STV_{LV} quantification for this experiment. * $p<0.05$ *vs.* baseline.

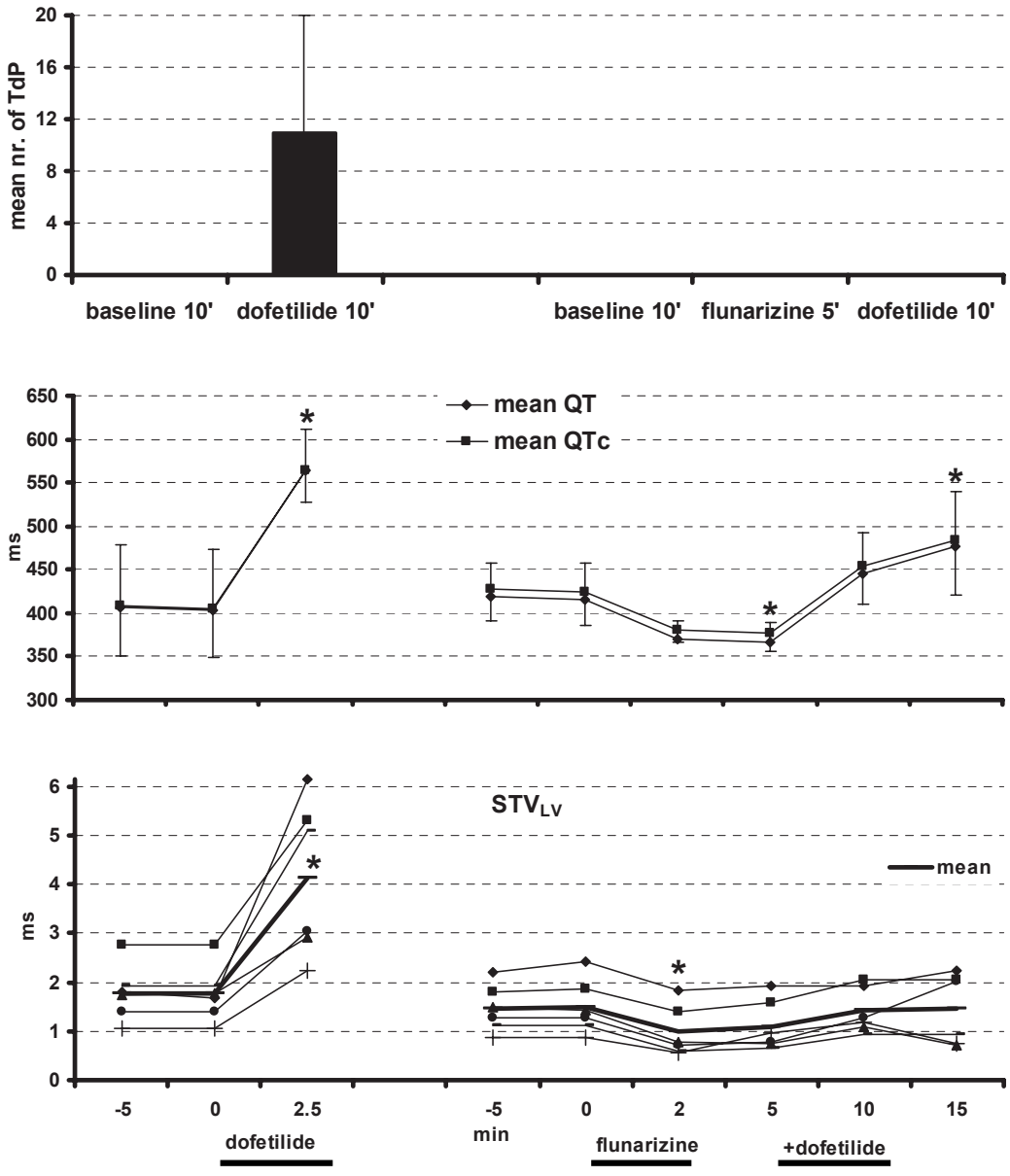

Figure 2. TdP prevention (upper panel) with flunarizine is presented in two serial experiments, first dofetilide alone (left) and with flunarizine pretreatment (right). The effects on QT/QTc (middle part) and STV_{LV} in individuals as well as average (lower part) are plotted.

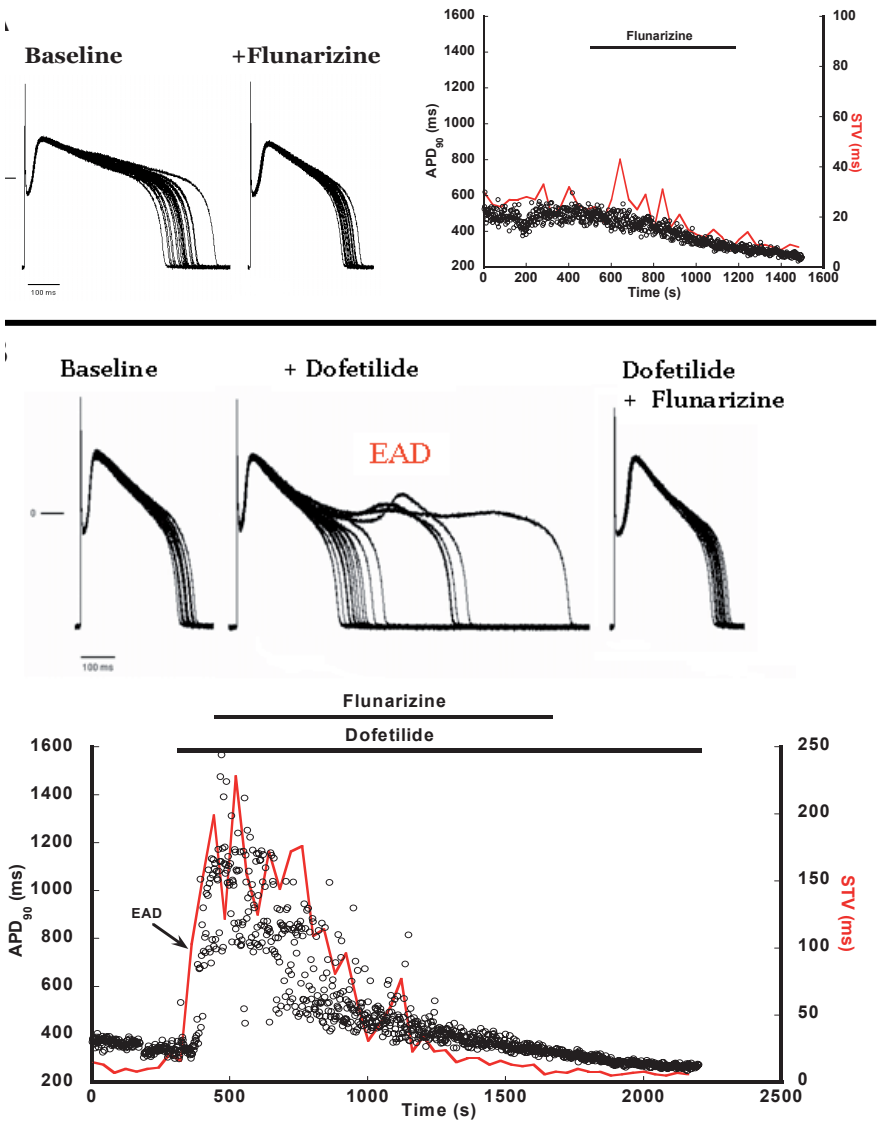

Figure 3. Anti-arrhythmic effects of flunarizine in isolated CAVB ventricular myocytes are depicted. **A:** 20 superimposed consecutive APs in baseline (left) and after flunarizine (middle) as well as the time course of APD (open dots) and STV_{APD} (as a line), baseline and with flunarizine perfusion are shown. **B:** Similar, 20 superimposed APs in baseline (left), with dofetilide induced EADs (middle) and after EADs suppression with flunarizine (right) and the temporal behavior of APD and STV_{APD} are shown for this experiment.

In dofetilide treated cells, APD increased from 337±119 to 507±153 ms ($p<0.05$) and STV from 14±14 to 65±34 ms ($p<0.05$). EADs occurred in 8 from a total of 9 cells. Addition of flunarizine suppressed all dofetilide-induced EAD's (8/8) and reversed APD (289±60 ms) and cellular STV (11±5ms) to baseline values. A representative example is shown in Figure 3B.

2) Antiarrhythmic effects of verapamil:

a. Verapamil suppression

Similar arrhythmia was seen with dofetilide in this group: TdP induction after 3.7±1 minutes with a median duration of 7.1 s. All TdP and MEBs were suppressed, while some SEBs remained in 3 dogs (Table 2, upper part). Verapamil did not affect the dofetilide-prolonged repolarization duration (QT, QTc, LVMAPD and RVMAPD) but reduced the variability of repolarization STV_{LV} to a level similar to control (Table 2, upper part).

b. Verapamil prevention

Verapamil pretreatment also prevented TdP induction remarkably, only one self-terminating TdP was seen. However, dofetilide was still able to generate numerous SEBs and few MEBs (Table 2, lower part). Verapamil did neither change baseline EP parameters, nor STV_{LV}. The duration of repolarization parameters (QT, QTc, LVMAPD and RVMAPD) was prolonged after adding dofetilide despite verapamil pretreatment. However the variability of repolarization (STV_{LV}) was not significantly increased after dofetilide (2.3±1.4 ms at 5 min., Table 2, lower part).

3) Insights into the mode of action

a. Effects of flunarizine and verapamil on I_{Na}

Figure 4 shows the effect of flunarizine (Fig. 4A, left) and verapamil (Fig. 4B, right) on late I_{Na} induced by ATX-II (Fig, 4, gray lines). Flunarizine (1 µM, Fig. 4A, red line) inhibited late I_{Na} by 94.37±2.33%, n=5 cells). However, at a concentration 10x higher flunarizine (10 µM) had minimal effect on peak I_{Na} (tonic block; 4.29±3.04%, n=4 cells). In contrast to flunarizine, verapamil (Fig. 4B, blue line, 10 µM) had no effect on either late I_{Na} (n=5 cells) or peak I_{Na} (tonic block; 10 µM, n=6 cells and 30 µM, n=4 cells).

b. Ca^{2+} sparks study

Acute application of these drugs on the frequency of spontaneous Ca^{2+} sparks in cardiac myocytes expressing a gain-of-function mutation in the RyR2 was examined. Flunarizine (1 µM) did not change the frequency of spontaneous Ca^{2+} sparks in RyR^{R4496C} cells (Fig. 5A, control 4.7±0.8, flunarizine 4.9±1.1 Ca^{2+} sparks/s/100 µm). In contrast, verapamil (10 µM) significantly reduced spontaneous Ca^{2+} spark activity by ≈35% (Fig 5B, control 4.9±0.5, verapamil 3.2±0.4 Ca^{2+} sparks/s/100 µm).

Table 2

	Baseline 1	Dofetilide	Verapamil
RR	1361±190	1520±210 *	1476±166
QT	456±67	611±92 *	557±97 *
QT_c	424±62	566±87 *	516±90 *
LV MAPD	349±88	505±110 *	466±95 *
RV MAPD	305±63	446±135 *	392±108 *
∆MAPD	44±39	72±36	92±92
STV_{LV}	1.7±0.4	3.2±1.1 *	1.5±0.7 $
TdP	0±0	9±5 *	0±0
MEB	0±0	9±4 *	0±0
SEB	1±1	50±31 *	9±15
	Baseline 2	**Verapamil**	**Dofetilide**
RR	1285±202	1212±228	1464±240 #
QT	442±71	436±57	651±47 * #
QT_c	417±58	417±41	611±34 * #
LV MAPD	332±68	328±34	554±77 *#
RV MAPD	324±43	318±37	545±53 *#
∆MAPD	32±29	33±16	30±16
STV_{LV}	1.3±0.4	1.4±0.6	2.3±1.4
TdP	0±0	0±0	0.2±0.4
MEB	0±1	0.2±0.4	3±6
SEB	2±4	1±2	28±44

Maximal effects of verapamil in suppression experiments (at 10 minutes, upper part) and at the end of the infusion in prevention experiments are shown (lower part). All EP parameters are expressed in ms and arrhythmias as average number per time interval. *, $p<0.05$ vs. baseline; $ $p<0.05$ vs dofetilide; # $p<0.05$ vs. verapamil pretreatment.

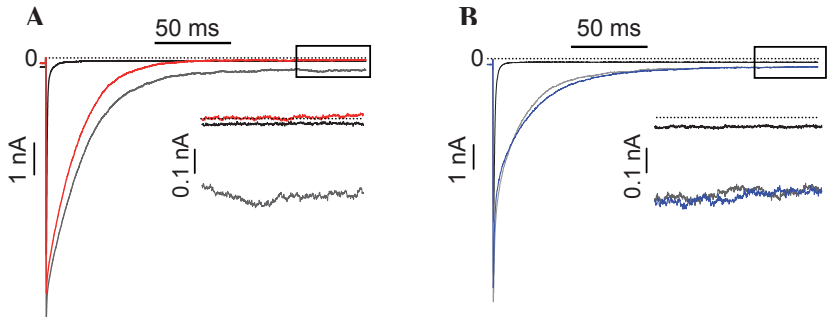

Figure 4. Effects of flunarizine (left) and verapamil (right) on late I_{Na}**:** representative recordings of late I_{Na} from a single cell in the absence of drug (control, black lines), during superfusion with 3 nM ATX-II (ATX-II, grey lines) and during superfusion with 1 μM flunarizine (left, red line) or 10 μM verapamil (right, blue line). Insets: expanded traces (last 50 ms following depolarizing pulse) of late I_{Na} in the absence (solid line) and presence of flunarazine (lred line) or verapamil (blue line), respectively.

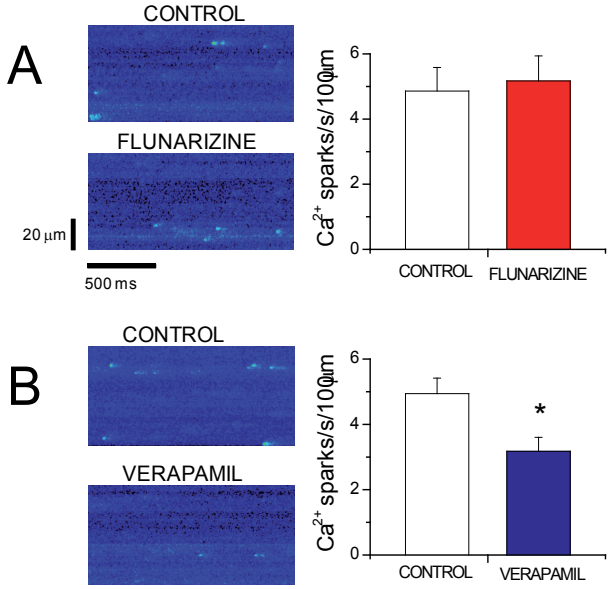

Figure 5. A: Left, representative line-scan images of spontaneous Ca^{2+} sparks recorded in a $RyR2^{R4496C+/+}$ ventricular myocytes in the absence (top) or presence (bottom) of 1 μM flunarizine. Right, Ca^{2+} spark occurrence before (white bar) and during (red bar) flunarizine (n=8 cells)

B: Similar, images of spontaneous Ca^{2+} sparks in the absence (top) or presence (bottom) of 10 μM verapamil. Right panel shows the average the average data in control (white bar) and with verapamil (blue bar, n=11 cells). *, $p<0.05$ *vs*. control.

c. In vivo effects of verapamil in combination with lidocaine

To verify if STV_{LV} reduction in baseline by flunarizine was in part due to inhibition of late I_{Na}, the effects of verapamil combined with lidocaine were explored. By the combination of these drugs repolarization duration was shortened (QTc from 353±35 to 306±21 ms) and STV_{LV} was reduced, effects similar to those seen with flunarizine alone (Figure 6).

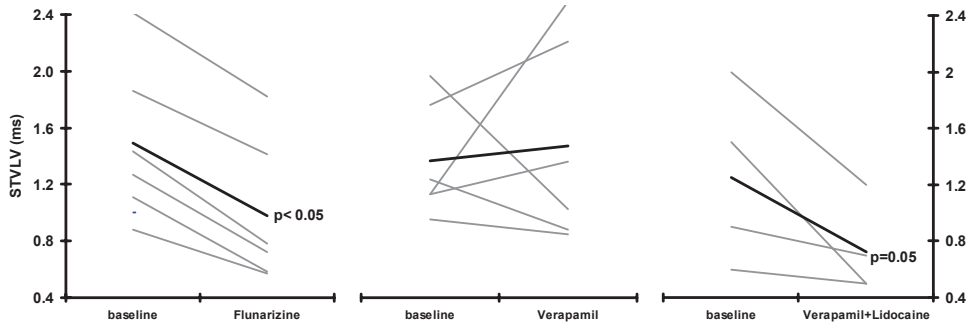

Figure 6. Effects of flunarizine (left), verapamil (middle) and combination verapamil-lidocaine on baseline STV_{LV}. Effects of these drugs on baseline STV_{LV} is pictured in individuals (thin lines) as well as a mean (thick line).

Discussion

The most important findings of this study can be summarized as follows: 1) Both Ca^{2+}-antagonists flunarizine and verapamil were equally and markedly effective in suppressing and preventing dofetilide-induced TdP, 2) this anti-arrhythmic effect was reflected in STV_{LV}, but not QT or LV MAPD, 3) flunarizine but not verapamil decreased BVR in baseline, which could be ascribed to its additional late I_{Na} blocking effect, and 4) verapamil reduced Ca^{2+} sparks, an effect not seen with flunarizine.

Ventricular arrhythmias

The shared Ca^{2+}-antagonism of verapamil and flunarizine was applied to investigate whether they could improve repolarization reserve reflected in protection against dofetilide-induced TdP. Repolarization dependent arrhythmias like drug-induced TdP normally occur in the setting of a prolonged QT-time under conditions that repolarization reserve is "challenged beyond capacity". Besides congenital long-QT, TdP can occur also in acquired long-QT syndromes. There is also evidence that congestive heart failure (CHF) induced electrical remodeling causing VTs and sudden cardiac death, is based upon a diminished repolarization reserve[31]. Lately it has been suggested that this reserve can be estimated by BVR. Supporting evidence comes from: a) individuals with an increased baseline BVR are at high risk

to sudden cardiac death, including CHF patients[19,32], b) elevated baseline BVR is related to the magnitude of electrical remodeling[33] and c) a further drug-induced increase in BVR precedes TdP arrhythmias while no increase in BVR confirms safe medication[20]. In addition, BVR was increased prior to I_{Kr} blockers-induced EADs in isolated myocytes from CAVB dogs[21,34].

The initiation mechanism for TdP in long QT syndromes involves EADs and EADs dependent triggered activity[23,35]. To develop new anti-arrhythmic drugs, it is important to understand possible targets that are key-players in the generation of EADs. Several have been identified, like L-type Ca^{2+} channel, the Na^{+} channel (with peak and late I_{Na}), ryanodine receptor and its regulating unit calstabin2 (FKBP12.6), SERCA2 with its regulatory unit phospholamban, calcium/calmodulin-dependent protein kinase II (CaMKII) and NCX. EADs may have distinct ways to be generated: 1) window currents, either through the $I_{Ca,L}$ or late I_{Na}, and 2) increased SR calcium load and abnormal Ca^{2+} release from the SR induced by NCX mediated inward currents. Especially $I_{Ca,L}$ has been studied extensively and proven to be relevant. Not only was it shown that $I_{Ca,L}$ block by verapamil or nitrendipine could prevent EADs from developing[8,36], but also that regional differences in the expression levels of L-type Ca^{2+} channels has implications for the origin of EADs[37]. This effect may also explain why I_{Kr} blockade by verapamil[17] and flunarizine are not pro-arrhythmic. An additional block of $I_{Ca,L}$ protects the heart from developing EADs despite I_{Kr} block induced QT lengthening[24]. This balance between $I_{Ca,L}$ recovery and ventricular repolarization serves also as a physiological stabilizer[38].

The second theory, the involvement of abnormal SR Ca^{2+} release in generating EADs, is more controversial[14,16]. Nevertheless there is evidence that DADs and EADs may occur in the same preparation[14,36,39,40], suggesting a possible similar etiology. Moreover, EADs and calcium transients have been related[41]. Indirectly, activation of CaMKII due to an increase in $[Ca^{2+}]_i$ might facilitate both $I_{Ca,L}$ and I_{Na} inducing EADs and SR release inducing DADs[42]. Due to methodological limitations, the relevance of this alternative (for the conditional phase) or the effects of drugs on this theory are difficult to investigate. However, investigating diastolic Ca^{2+}-sparks known to underlie DADs generation is an interesting approach to address the question, whether flunarizine and verapamil inhibit disturbed SR Ca^{2+} release events.

Calcium antagonism

Pharmacologic antagonism at cardiac L-type Ca^{2+} channels is limited to three classes of drugs: phenylalkalamines (verapamil), benzothiazepines (diltiazem) and dihydropiridines (nifedipine). Flunarizine belongs to a different category of Ca^{2+}-antagonists. Clinically, the drug has been used to treat neurological disorders, such as migraine and has been termed as calcium overload blocker[43]. The latter implies that flunarizine may have an intracellular target. However, until now, only sarcolemmal effects have been described. Besides blocking three type of Ca^{2+}-channels[44,45], $I_{Ca,L}$ (IC_{50}=4.6-10 µM), $I_{Ca,N}$ (0.8 µM) and $I_{Ca,T}$ (3.3-11 µM), flunarizine is also a potent I_{Kr} blocker (5.7 nM) and I_{Ks} (0.7 µM)[18].

Verapamil is known to block $I_{Ca,L}$ (0.6-15.5 μM)[46], I_{Kr} (0.1 μM)[17] and IKs (5.7-6.3 μM)[11]. According to the producer, our dose of flunarizine will reach a total plasma concentration around 1.7 μM (828ng/ml, MW 477.4), while this is for verapamil around 0.5 μM[47].

In susceptible dogs with CAVB, both drugs were very effective (100% efficacy) in preventing and terminating dofetilide-induced TdP. They were much stronger than other drugs such as the late I_{Na} blockers ranolazine and lidocaine, which were effective in approximately 60% of the animals, whereas the $I_{K,ATP}$ agonist levcromakalim was slightly more effective (70%, unpublished data). This confirms that inhibition of $I_{Ca,L}$ is a very effective way to treat dofetilide-induced TdP assuming that no other actions are involved (see below).

Mode of action

The anti-arrhythmic potential of flunarizine and verapamil was clearly reflected by the changes in STV_{LV}. Its suppressive actions were associated with a reduction in STV_{LV}, whereas the preventive effects could be seen in keeping STV_{LV} at low(er) levels. Anti-arrhythmic properties of flunarizine were confirmed *in vitro* on drug-induced EADs and cellular BVR. Thus, BVR is indicative for the ability of the heart to withstand a proarrhythmic challenge. However, the action on the other electrophysiological parameters differed. Flunarizine showed a pronounced action on repolarization parameters such as $QT_{(c)}$ and LV MAPD and cellular APD, whereas the effect of verapamil on repolarization time was much smaller or even absent.

Secondly, flunarizine decreased baseline STV_{LV}, suggesting that this drug may increase repolarization reserve. This interpretation is consistent with the greater effect of verapamil combined with lidocaine on STV_{LV} (figure 6) and LV MAPD than verapamil alone.

The fact that flunarizine decrease APD/QTc while verapamil does not could in part contribute to the mechanism by which flunarizine reduces STV_{LV} or STV_{APD}. However the contribution of APD to STV_{LV} is not seen in the suppression experiments where verapamil shortened STV without a significant reduction in APD/QTc.

In order to gain more insight in the mode of action of these drugs, we undertook cellular experiments to determine their possible action against late I_{Na} and Ca^{2+} sparks. It was shown that flunarizine but not verapamil blocked late I_{Na}. This could explain why flunarizine, but not verapamil, was effective against veratridine-induced contractures[48]. On the other hand, verapamil but not flunarizine could reduce the frequency of diastolic Ca^{2+} sparks in a cell model expressing a gain-of-function mutation in the RyR2 (also termed as RyR2 Ca^{2+} leakage). These results show that the antiarrhythmic molecular mechanism of verapamil and flunarizine could involve different targets. Regarding flunarizine it is possible to discard an action of flunarizine on RyR2 activity. As mentioned, there is controversy concerning the paradigm that SR calcium leak may indirectly provide inward currents that contribute to EADs induction[41,49]. One way to study this is by application of drugs that specifically block this release. Ryanodine and K201 are drugs that block the RyR2, although not with a high degree of specificity. When evaluating the literature concerning ryanodine and its action on DADs or EADs, it becomes apparent that the results are not consistent. Ryanodine is known to be anti-arrhythmic against DADs and DAD-dependent VT[36,39]. In dogs with

CAVB, ryanodine (10 mg) was effective against drug-induced TdP, whereas ryanodine was not effective against Cesium or ATX-II induced EADs[6,36,40], but anti-arrhythmic against catecholamine-induced EADs[39]. Ryanodine and flunarizine were both effective against acceleration induced EADs[50]. Until there is a specific blocker for unconditional Ca^{2+} leak, it will be difficult to prove SR leakage to be part of the EAD generation. It is evident however, that adding blocking properties against either late I_{Na} or Ca^{2+} sparks could generate more anti-arrhythmic "strength". Future studies are necessary to evaluate which of the two additional actions is the most attractive.

In conclusion, a robust anti-arrhythmic efficacy was seen with flunarizine and verapamil. This suppressive and preventive action of the drugs was reflected in STV_{LV} or cellular STV_{APD}. Their different electrophysiological response may be related to different additional effects of the two drugs: flunarizine blocks late I_{Na}, whereas verapamil reduces Ca^{2+}-sparks.

Acknowledgements

The study was supported by a grant from the EU FP6 (LSHM-CT-2005-018802, Contica), and by a Veni grant from The Netherlands Organization for Scientific Research (916.56.145) to G. Antoons.

Within the Contica framework we received the knock-in mice $RyR2^{4496C+/+}$ from Drs S. G. Priori and C. Napolitano, Pavia, Italy.

SR and LB are employees of CV Therapeutics.

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Chapter 6

Late Na^+ current inhibition by ranolazine reduces Torsades de Pointes in the chronic AV block dog model

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Accepted for publication, J Am Coll Cardiol, 2009

Abstract

Introduction: Ranolazine is an anti-anginal drug that exerts its action mainly through inhibition of the late Na^+ current, I_{NaL}. Despite its I_{Kr} blocking properties, ranolazine is effective against arrhythmias in LQT3 syndromes. We investigated whether ranolazine reduces dofetilide-induced Torsades de Pointes (TdP) in a model of acquired LQT with downregulated K^+ currents due to hypertrophic remodeling, the dog with chronic atrioventricular block (CAVB).

Methods and Results: Ranolazine was administered to CAVB dogs before or after TdP induction with dofetilide. After dofetilide, ranolazine reduced the number of TdP episodes from 10±3 to 3±1 (P<0.05), and partially reversed the increase of repolarization variability (BVR) with no abbreviation of the dofetilide-induced QT prolongation. Likewise, pretreatment with ranolazine, or using lidocaine as specific Na^+ channel blocker, attenuated TdP, but failed to prevent dofetilide-induced increases in QT, BVR and ectopic activity. In single CAVB myocytes, ranolazine suppressed dofetilide-induced early afterdepolarizations (EADs) concentration-dependently: in 25% of cells at 5 µmol/L, in 75% at 10 µmol/L and in 100% at 15 µmol/L. At 5 µmol/L, ranolazine blocked 26±3% of TTX-sensitive I_{NaL}, and 49±3% at 15 µmol/L. Despite smaller amplitude of I_{NaL} in CAVB (0.08±0.01 pA/pF, vs. 0.173±0.03 pA/pF in control, P<0.05), full I_{NaL} inhibition by 5 µmol/L TTX equally shortened action potential duration (29±3% in CAVB and 28±4% in control, NS), and completely abolished dofetilide-induced EADs.

Conclusions: Despite downregulation of I_{NaL} in remodeled CAVB hearts, ranolazine is antiarrhythmic against drug-induced TdP. The antiarrhythmic effects are reflected in concomitant changes of BVR

Introduction

The voltage-dependent sodium channels produce a fast inward current upon depolarization that marks the initial upstroke of the cardiac action potential (AP). Following activation, most channels rapidly inactivate, but some sustained activity remains: the late sodium current, I_{NaL}[1]. This current is upregulated in heart failure, where it contributes to AP prolongation in failing myocytes[2,3]. Inhibition of I_{NaL} abolished early afterdepolarizations (EAD) in these cells[4]. A link between enhanced I_{NaL} and proarrhythmia has been revealed by our understanding of the biophysical basis of the LQT3 syndromes: gain-of-function mutations in the Na^+ channel induce torsades de pointes (TdP) arrhythmias in these patients[5]. These findings have renewed our interest in Na^+ channel blockers as potential anti-arrhythmic strategy against arrhythmias. Ranolazine is an anti-anginal agent that has been explored recently for its potential as an anti-arrhythmic agent because of its I_{NaL} blocking properties. This block is more sensitive than for the fast component of the Na^+ current[6] and may be of particular importance in heart failure where conduction is already compromised[7]. Ranolazine also blocks I_{Kr}[8,9], and prolongs QT in patients[10], but is not pro-arrhythmic[8,9,11] and shortens QT and suppresses arrhythmias in LQT3 syndrome[12-14]. Likewise, ranolazine stabilized repolarization in failing myocytes with prolonged repolarization and upregulated I_{NaL}[15]. Interestingly, ranolazine has been proven anti-arrhythmic in LQT syndromes caused by mechanisms other than abnormal Na^+ channel activity[16,17]. Ranolazine also lowered incidence of arrhythmias in patients who survived an acute coronary syndrome[18], and first reports in patients with AF are promising[19].

The dog with chronic atrioventricular block (CAVB) is a well-characterized model of proarrhythmia. Its high susceptibility to TdP relates to abnormal repolarization due to remodeling. This includes downregulation of repolarizing K^+ currents, and upregulation of an inward Na/Ca exchange current[20-23]. In the present study, we tested the effect of ranolazine on suppression and prevention of TdP induced by dofetilide, a selective I_{Kr} blocker. We linked this to electrophysiological parameters, including beat-to-beat variability of repolarization (BVR) because of its high predictive value of TdP risk[24]. For comparison, we also tested lidocaine, a I_{Na} blocker with less selectivity for I_{NaL} over the fast component of I_{Na}[25], but with no or much weaker inhibitory effects on I_{Kr}[26]. In single myocytes, we characterized the functional properties of I_{NaL}, and we determined the effects of ranolazine on I_{NaL} and dofetilide-induced EADs.

Methods

Animal handling was in accordance with the European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EU). The experiments were approved by the Utrecht University Committee for Experiments on Animals.

In vivo studies

Thirty-two experiments were performed in fourteen adult mongrel dogs with CAVB (24±3 kg body weight, 11 females, Marshall, USA). AV block was created by radiofrequency ablation as described in detail elsewhere[27]. Experiments were performed 4 weeks after AV block when electrical remodeling is completed[27]. Details concerning anesthesia, animal care, data collection and analysis have been described previously[28]. After premedication (0.5 mg/kg methadone, 0.5 mg/kg acepromazine and 0.5 mg atropine I.M.), anesthesia was induced by sodium pentobarbital (25 mg/kg I.V.) and maintained by 0.5% halothane in a mixture of O_2 and N_2O (1:2). For the prevention studies (n=18), animals received pentobarbital in combination with 1.5% isoflurane.

To induce TdP, CAVB dogs received a dose of 0.025 mg/kg dofetilide over 5 min or until TdP occurred within this period (Fig 1A). Non-inducible dogs (n=3) were excluded from further study. Ranolazine (CV Therapeutics, Palo Alto, USA) was given 10 min after the start of dofetilide as a bolus of 4 mg/kg in 30 s followed by infusion at a rate of 0.225 mg/kg min^{-1} for 10 min to 5 animals (Fig 1A). A second group of 6 dogs received lidocaine (B. Braun Melsungen AG, Germany) at a dose of 3 mg kg^{-1} in 2 min.

Six TdP-inducible CAVB dogs were used for serial testing to determine the effect of ranolazine and lidocaine to prevent TdP induction (Fig 2A).

Blood samples were collected from a venous catheter every 5 min during experiment. Heparin-treated samples were centrifuged at 4000 rpm at 4 ºC and stored at -80 ºC for further analysis. Concentrations of ranolazine were determined using high-performance liquid chromatography. Analysis was performed at CV Therapeutics, Palo Alto, USA.

Cellular experiments

For cell isolation, hearts were quickly excised from anesthetized CAVB dogs. Age-matched dogs with normal sinus rhythm served as controls. Cells were enzymatically digested from the midmyocardial layer of the ventricles, as previously described[31]. Action potentials and whole-cell Na^+ currents were measured using the patch-clamp technique; Action potentials were recorded with an Axopatch 200B amplifier under whole-cell current-clamp using the perforated patch technique. Patch pipettes had a resistance of 1-3 MΩ when filled with internal solution (in mmol/L): 130 KCl, 10 HEPES, 5 MgATP, 0.5 $MgCl_2$, 10 NaCl, 1 $CaCl_2$, 0.00026 amphotericine B, pH adjusted to 7.20 using KOH. External solution contained (in mmol/L): 137 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 11.8 HEPES and 10 glucose, pH adjusted to 7.40 with NaOH. Action potentials were elicited with 2-ms current injections at a stimulation frequency of 0.5 Hz. Transmembrane potentials were low-pass filtered at 2 kHz and sampled at 4 kHz using a Digidata 1200 analog-to-digital converter and PClamp 9 software (Axon instruments Inc.).

Whole-cell Na^+ currents were measured using standard ruptured patch configuration. In the external solution, K^+ was replaced by Cs^+ to avoid contamination of K^+ currents; nifedipine (20 µmol/L) was added to block Ca^{2+} currents. Pipettes were filled with (in mmol/L): 10 NaCl, 120 CsCl, 20 TEA-Cl, 5 MgATP, 5 EGTA and 5 HEPES; pH was adjusted to

7.2 with CsOH. Currents were filtered at 2 kHz and sampled at 10 kHz. The persistent component of the Na^+ current, I_{NaL}, was measured as the TTX-sensitive current elicited by 500 ms depolarizing pulses from -130 mV to -40 mV. Interval between pulses was 30 s. The current amplitude was calculated by subtracting a current trace recorded in the presence of 5 µmol/L TTX from a trace under baseline, and was measured at the end of pulse. Membrane currents were normalized to cell capacity (pA/pF).

Tetrodotoxin was prepared as a 10 mmol/L stock solution in water and diluted to a final volume of 5 µmol/L. Ranolazine was made as a 15 mmol/L stock solution in water. Dofetilide 10 mmol/L stock solutions were prepared in DMSO and diluted: 10000 before use. Solutions were made freshly for each day of experiments. All experiments were done at 37 ºC.

Statistics

Data are expressed as mean ± S.E.M. For comparisons between groups, unpaired Student's t-test was used. For dependent measurements, ANOVA for repeated measurements was used with Bonferroni post-hoc testing. Friedman ANOVA was used as non-parametric equivalent when normality test failed. Statistical analysis was performed in SigmaStat 3.10 (Systat Software, Inc.). Values of $P < 0.05$ were considered significant.

Results

Ranolazine reduces proarrhythmic activity in CAVB dogs

Fig 1A shows a representative example of the ventricular rhythm at baseline (left), after dofetilide (middle) and with ranolazine (right). Dofetilide prolonged QTc, increased STV and induced TdP (Fig 1B & C). Ranolazine significantly reduced the number of TdP episodes (Fig 1B). This was associated with a reduction of STV, while QT_c remained prolonged (Fig 1C). Proarrhythmic activity was not completely suppressed by ranolazine, as evidenced by the presence of multiple ectopic beats (mEBs) and TdP episodes (Fig 1A & B) remaining in 4/5 dogs. Plasma levels of ranolazine reached 15.7±0.6 µmol/L at 10 min (n_{dogs}=3), comparable to values reported in a previous study with larger sample size[11].

In prevention experiments (Fig 2A), ranolazine plasma levels reached a plateau at 5 min infusion and remained constant throughout the experiment; concentration was 20±3 µmol/L at 15 min (Fig 2B). Ranolazine alone did not produce significant changes in QTc or STV (Fig 2C). Despite pre-administration of ranolazine, dofetilide prolonged QTc and tended to increase STV, but the latter increase was no longer significant (Fig 2C and Table). Ranolazine reduced the number of dofetilide-induced TdP episodes (Fig 2D). Albeit less frequently and of shorter duration, TdP was still seen in 4/6 dogs (Fig 2D). TdP duration was shortened from 11±2 to 5±2 s ($P<0.05$). Single and multiple EBs occurred in the majority of animals (5/6).

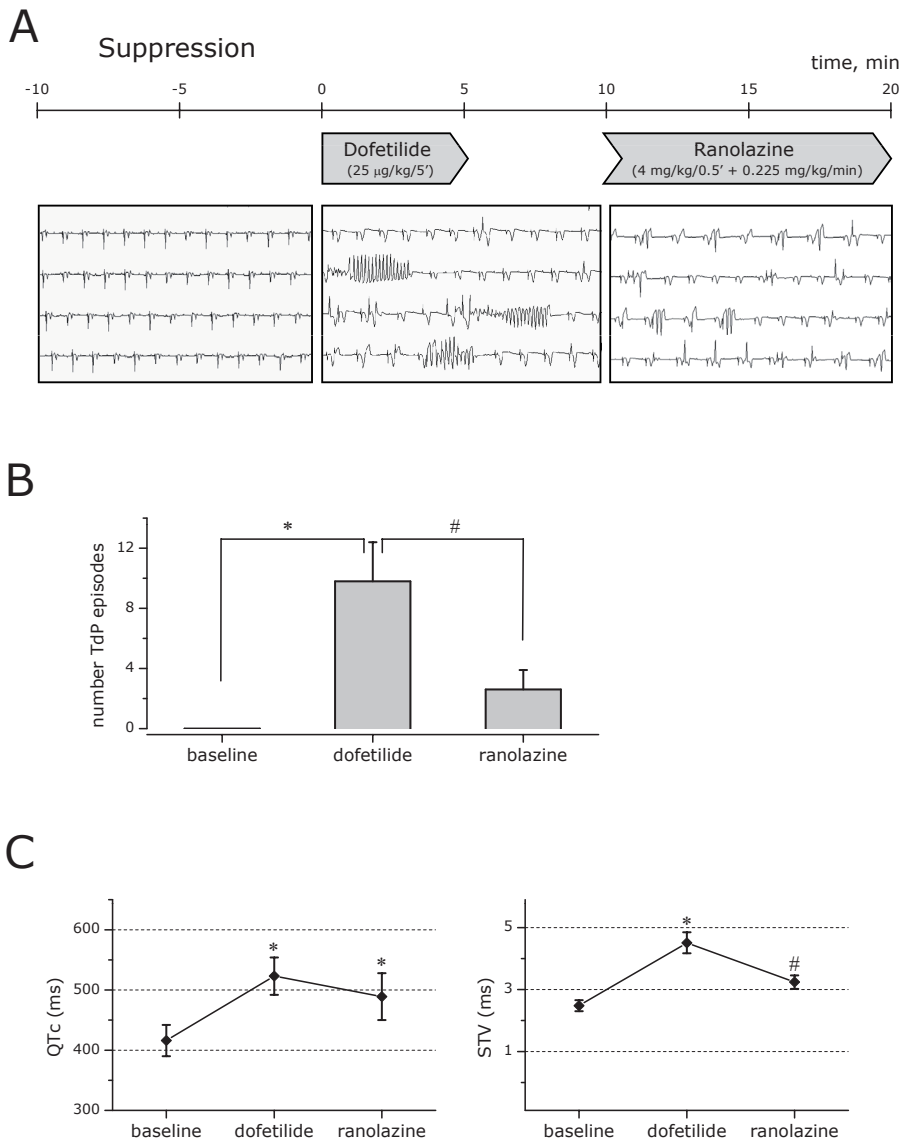

Figure 1. Suppression of dofetilide-induced TdP in CAVB dogs by ranolazine

A, Representative ECG trace (lead II) under baseline (left), after dofetilide (middle) and during ranolazine (right) administration showing dofetilide-induced TdP episodes (middle) and suppressive effects of ranolazine (right). Note the presence of mEBs during ranolazine infusion. **B**, Number of TdP episodes counted over a 10-min period under baseline and after drug administration; averaged data from 5 CAVB dogs. **C**, Pooled data for QTc interval (left panel) and short-term variability (STV, right panel). Parameters were measured just before the first extrasystole during dofetilide and 10 min after ranolazine infusion (n_{dogs}=5, * indicates P<0.05 vs. baseline, and # vs. dofetilide).

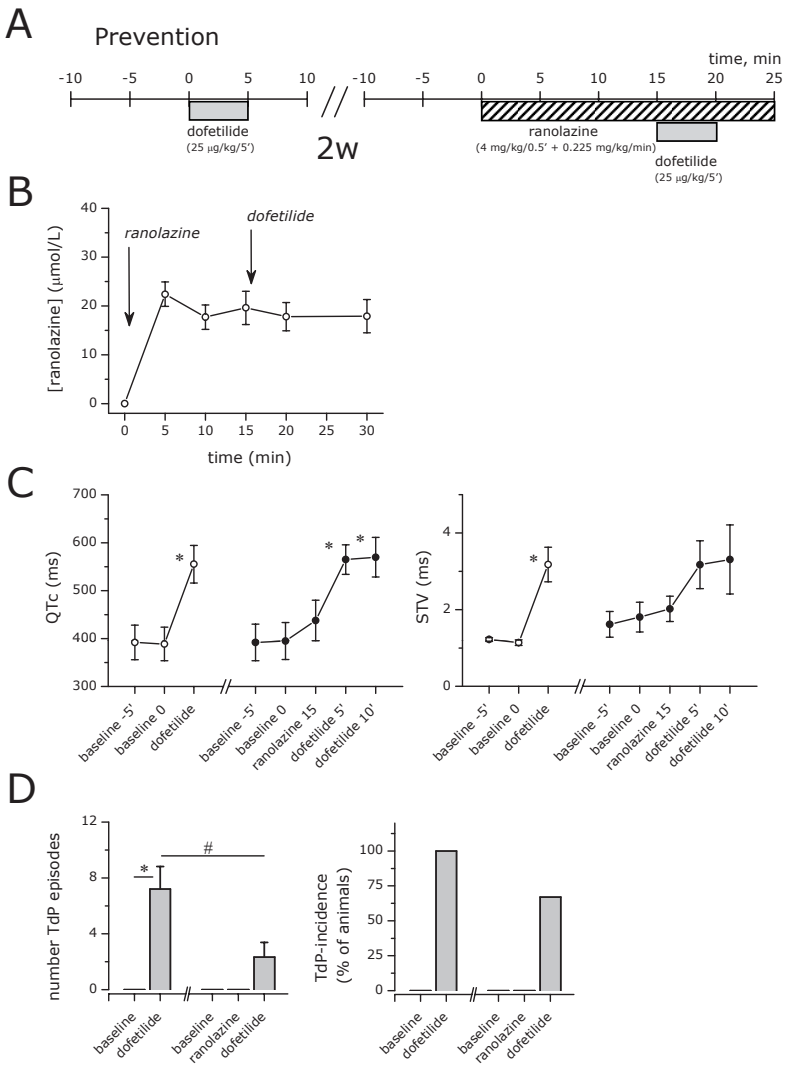

Figure 2. Prevention of TdP after ranolazine pretreatment

A, Protocol for serial testing of TdP inducibility before and after ranolazine pretreatment. In the control experiment, dofetilide was given for 5 min or until TdP occurred within this period. Same dose and infusion time were used in the subsequent experiment after a 15-min administration of ranolazine. Time between experiments was 2 weeks. **B**, Plasma concentration of ranolazine. Arrows indicate the start of the 15-min ranolazine and 5-min dofetilide infusion. **C**, Dofetilide-induced changes in QTc interval (left panel) and STV (right panel), before and after ranolazine administration (open vs. closed circles). * indicates P<0.05 vs. baseline at 5 min before the start of infusion, and # vs. dofetilide. **D**, Number of dofetilide-induced TdP episodes (left) and incidence (% of inducible dogs, right panel) after ranolazine pretreatment (P*<0.05). Data are from 6 CAVB dogs.

Table. Electrophysiological and proarrhythmic properties of dofetilide before and after ranolazine and lidocaine treatment

	Baseline 1	Dofetilide 1	Baseline 2	Ranolazine	Dofetilide 2	Baseline 3	Lidocaine	Dofetilide 3
RR, ms	1226±148	1254±155	1574±102	1676±74	1904±141	1499±133	1481±129	1481±129
QT, ms	412±29	577±36*	442±46	497±45	644±33†‡	425±41	360±25†	627±41†‡
QTc, ms	392±36	555±39*	392±38	438±43	565±31†‡	382±34	318±20†	586±38†‡
LV MAPD, ms	334±27	525±34*	362±49	410±49	573±56†‡	323±41	281±25	522±51†‡
RV MAPD, ms	274±21	361±23*	330±24	384±24	503±41†‡	270±17	247±14	352±25†‡
∆MAPD, ms	54±4.3	147±27*	53±14	47±15	73±26	53±29	36±11	169±44†‡
LV STV, ms	1.2±0.1	3.1±0.6*	1.6±0.3	2.0±0.3	3.3±0.9	1.4±0.3	1.2±0.2	3.1±0.4†‡
single EBs	6±3	62±18*	1±0.4	1±1	84±38	1±0.3	0	27±14
multiple EBs	0	21±8*	0	0	6±3	0	0	2±1$
nr TdPs	0	7±2*	0	0	2±1$	0	0	1±1$
TdP incidence	0	6/6	0	0	4/6	0	0	3/6

Serial electrophysiological and proarrhythmic properties of dofetilide before and after ranolazine - lidocaine treatment
Six CAVB dogs received a dofetilide infusion in 3 serial experiments; in the 2nd and 3th experiment, a 15-min ranolazine or 2-min lidocaine infusion was given prior to dofetilide. Parameters were measured before the first extrasystolic beat (dofetilide 1) or 10 min after infusion (dofetilide 2-3); ranolazine was analyzed at 15 min and lidocaine at 5 min after the start of infusion. There were no significant differences within the baseline data. * indicates $P<0.05$ vs. baseline 1; † vs. baseline 2-3; ‡ vs. ranolazine-lidocaine; $ vs. dofetilide 1.•

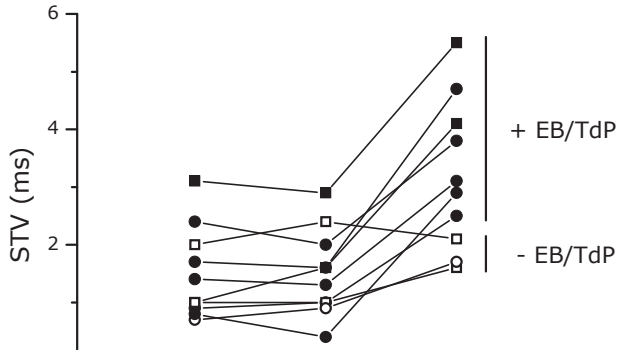

Figure 3. Relation between short-term variability and ectopic activity/TdP
Individual changes of short-term variability (STV) under baseline, during pretreatment with ranolazine (square symbols) or lidocaine (circles), and after dofetilide. Each line represents an individual animal. Data are taken from Table, and grouped according to ectopic beats and/or TdP occurrence (+ EB/TdP, closed symbols, n_{exp}=7; - EB/TdP, open symbols, n_{exp}=3).

TdP suppression and prevention by lidocaine

Suppression of dofetilide-induced TdP by lidocaine was studied in 6 animals using a similar protocol as described in Fig 1A for ranolazine. Results were comparable: lidocaine reduced the number of TdP episodes to 1.5±2 and TdP remained in 2/6 dogs. There was no shortening of the dofetilide-induced QTc prolongation (478±17 to 514±27 ms), but the antiarrhythmic effect was associated with a reduction of STV (3.7±1.2 ms with dofetilide vs. 2.3±0.4 ms with lidocaine, P<0.05).

The results of the prevention experiments with lidocaine are summarized in the Table. Lidocaine shortened QTc, but failed to attenuate dofetilide-induced QT prolongation, increases in MAP duration, interventricular dispersion, and STV. As with ranolazine, dofetilide-induced TdP episodes occurred less frequently in the presence of lidocaine, but were still observed in 3/6 CAVB dogs; mEBs were seen in 4/6 dogs.

Fig 3 summarizes individual data on STV according to arrhythmogenic outcome, defined as the occurrence of EBs or TdP. Effective prevention of arrhythmias either with lidocaine or ranolazine (n_{exp}=3) was associated with no change in STV, whereas an increase in STV preceded proarrhythmia (n_{exp}=7).

Late Na⁺ current is reduced in CAVB

The late component of the Na^+ current was measured as the current sensitive to 5 µmol/L TTX elicited by a 500 ms depolarizing step at -40 mV from a holding potential of -130 mV, in CAVB cells, and in control dogs (Fig 4A, left). The amplitude of the inward current was significantly reduced in CAVB: -0.08±0.01 vs. -0.173±0.03 pA/pF in control cells (Fig 4A,

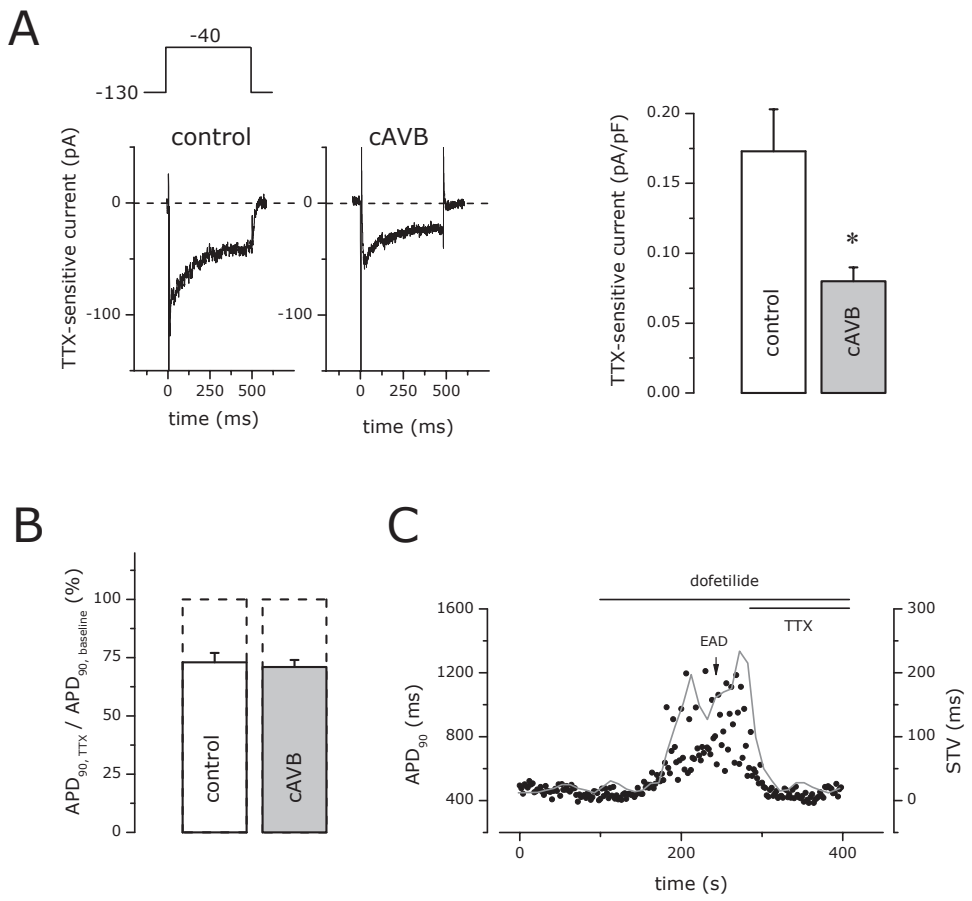

Figure 4. Late Na^+ current is reduced in CAVB

A, Left panel, TTX-sensitive currents elicited by a 500 ms depolarizing pulse from -130 to -40 mV in control vs. CAVB. Currents were obtained after substraction. Right panel, Pooled data of current densities (pA/pF) measured at the end of the depolarizing pulse in 6 control cells (n_{dogs}=3) and 12 CAVB cells (n_{dogs}=4, P<0.05). **B**, Relative AP duration, APD_{90}, in the presence of 5 mmol/L TTX as a percentage of baseline APD_{90} in CAVB (n=9) vs. control cells (n=8, NS). C, Time course of a typical experiment in a CAVB cell showing successive AP durations measured at 0.5 Hz (APD_{90}, circles) and STV values (grey line) under baseline, in the presence of dofetilide (1 mmol/L) and after addition of TTX (5 mmol/L). Addition of TTX started after the appearance of EADs with dofetilide (indicated by arrow).

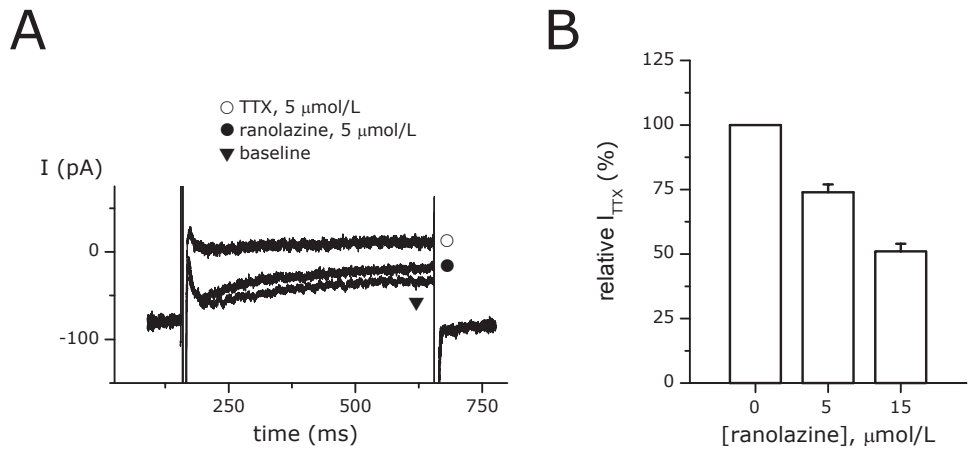

Figure 5. Inhibition of TTX-sensitive I_{NaL} by ranolazine in CAVB
A, Representative current traces measured during a 500 ms depolarizing step from -130 to -40 mV in a CAVB cell, under baseline, in de presence of ranolazine (5 μmol/L) and TTX (5 μmol/L). **B**, Relative amplitude of the current sensitive to 5 μmol/L (n=6) and 15 μmol/L ranolazine (n=8), as a percentage of the TTX-sensitive current in CAVB. Amplitudes were calculated by substracting the current traces in the presence of TTX and ranolazine from baseline currents.

right, P<0.05). Despite its smaller amplitude, the TTX-sensitive current equally contributed to the AP duration in CAVB: 5 μmol/L TTX shortened APD_{90} by 29±3% in CAVB (n=9) and by 28±4% in control (n=8, NS, Fig 4B).

Fig 4C shows the duration of successive APs and STV during the time course of a typical experiment where dofetilide was used to induce EADs which were typically preceded by AP prolongation and an increase of STV. Addition of 5 μmol/L TTX fully suppressed EADs, and reversed the dofetilide-induced increase of APD and STV to baseline values.

Suppression of afterdepolarizations by ranolazine is concentration-dependent

In CAVB dogs, total ranolazine concentrations were within a range of 7 to 30 μmol/L with ≈65% of total concentration bound[32], concentrations between 5 and 15 μmol/L were used. Fig 5A shows current traces measured in a CAVB cell during a step from -130 to -40 mV under baseline, and in the presence of 5 μmol/L TTX and ranolazine. The proportion of TTX-sensitive current blocked by ranolazine in CAVB was 26±3% at 5 μmol/L (n=6), and 49±3% at 15 μmol/L (n=8, Fig 5B).

Fig 6A shows typical recordings of APs and dofetilide-induced EADs in a CAVB cell, with in the lower panel changes in AP duration and STV. EADs were suppressed by 15 μmol/L (n_{cells}=9), although ranolazine did not shorten AP duration following dofetilide (P=0.27), or

significantly reduced STV at 15 µmol/L (P=0.13; Fig 6B). However, values were no longer different from baseline. In the particular example of Fig 6A, EADs were not suppressed by ranolazine when applied at 5 µmol/L. The concentration-dependent suppression of EADs by ranolazine is summarized in Fig 6C: in 25% of CAVB cells at a concentration of 5 µmol/L (n=4), vs. 75% at 10 µmol/L (n=4) and in 100% at 15 µmol/L (n=9).

Discussion

In the CAVB dog model, ranolazine significantly suppressed and prevented dofetilide-induced TdP arrhythmias. However, BVR was only slightly reduced, in line with the observation that proarrhythmic activity was not completely abolished. Lidocaine, a specific Na^+ blocker, had similar effects. An interesting finding of the present study was that full inhibition of I_{NaL} by TTX was sufficient to suppress dofetilide-induced EADs in single cells, despite the fact that I_{NaL} was downregulated in CAVB. Ranolazine abolished EADs but only at higher concentrations when there was substantial I_{NaL} block of ≈50%. Thus the incomplete antiarrhythmic activity of ranolazine is not due its I_{Kr} blocking properties, but more likely to a combination of reduced I_{NaL} and an insufficient degree of inhibition at therapeutic concentrations.

Remodeling of I_{NaL} in hypertrophy vs. heart failure

The CAVB heart develops compensated hypertrophy, and electrical remodeling includes changes in delayed rectifying K^+ currents and Na/Ca exchange[20,21,33]. Additionally, in the present study, we found a decrease of I_{NaL}. This is at odds with heart failure, where I_{NaL} is upregulated despite smaller peak currents[2] and contributes to prolongation and abnormal repolarization[4]. In CAVB, we previously reported lower expression levels of cardiac Na^+ channels together with a reduction of peak current[34].

Possible mechanisms underlying antiarrhythmic effects of ranolazine

The antiarrhythmic properties of ranolazine were reported at first in LQT3[13,14], and confirmed in failing myocytes with upregulated I_{NaL}[15]. Hence, the efficacy of ranolazine was ascribed to its I_{NaL} blocking properties. Interestingly, ranolazine was proven antiarrhythmic against EADs and TdP caused by mechanisms other than abnormal I_{NaL}, including enhanced Ca^{2+} channel activity mimicking LQT8[16], and through inhibition of I_{Kr} as a model for LQT2[8]. Reduced transmural dispersion as well as a decrease of repolarization variability were proposed as antiarrhythmic mechanisms[13,15,16]. BVR originates in the plateau phase of the AP and late Na^+ current may indeed contribute to variability[35]. In CAVB, ranolazine attenuated changes in dofetilide-induced BVR, but only to a limited extent.

Ranolazine prevents excessive Ca^{2+} loading of the cell by lowering Na^+ levels through inhibition of I_{NaL}[36]. In CAVB, Na^+ levels are high which enhances Ca^{2+} loading[22]. The subsequent increase of SR Ca^{2+} release may facilitate EADs by promoting inward Na/Ca exchange

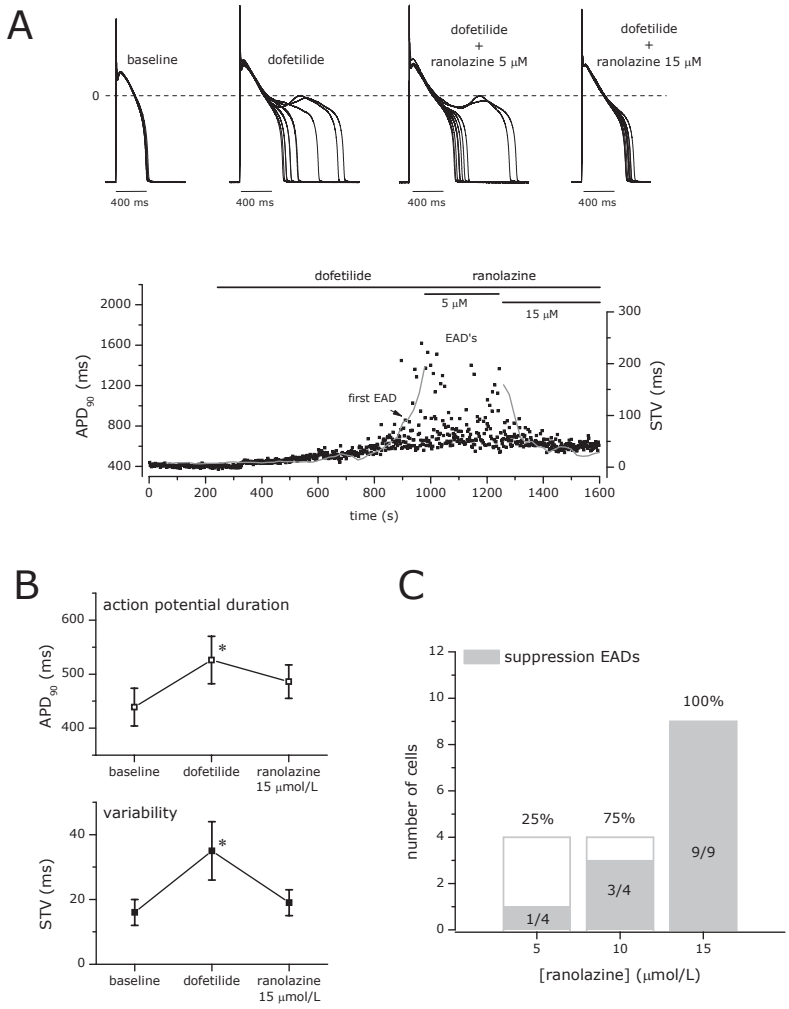

Figure 6. Ranolazine suppression is concentration-dependent

A, Original recordings of APs measured at 0.5 Hz in a CAVB cell where ranolazine was applied at a concentration of 5 and 15 μmol/L following dofetilide-induced EADs. The lower panel shows time-dependent changes in APD_{90} (symbols), STV (line) and EAD occurrence. EADs continued with ranolazine when applied at 5 μmol/L for approximately 4 min. Upon addition of 15 μmol/L ranolazine, EADs were completely suppressed within 2 min. **B**, Pooled data for APD_{90} (left panel) and STV (right panel) of 9 CAVB cells (n_{dogs}=5). For dofetilide, parameters were determined immediately prior to the first EAD (* indicates P<0.05 vs. baseline)

C, Proportion of CAVB cells showing EAD suppression at the indicated concentrations of ranolazine. The highest concentration of 15 μmol/L was applied in 9 cells. A subset of cells was used for additional application of 5 (n_{cells}=4) or 10 μmol/L ranolazine (n_{cells}=4).

current allowing Ca^{2+} window currents to develop, and more Ca^{2+} channels may be available for reactivation due to a larger recovery from release-dependent inactivation[21,37]. In addition, high Ca^{2+} loads favor spontaneous Ca^{2+} release and delayed afterdepolarizations[33]. By reversing the increased Na^+ levels, ranolazine may reduce triggered activity in CAVB.

Limited antiarrhythmic potential of Na^+ channel blockers in CAVB

Although TdP occurrence is less, ranolazine did not completely abolish ectopic activity. At the therapeutic range, ranolazine blocks I_{Kr}[8]. In the remodeled CAVB heart with reduced repolarization reserve, additional block of I_{Kr} could confound the antiarrhythmic effects of ranolazine through inhibition of I_{NaL}. Incomplete antiarrhythmic activity however was also seen with lidocaine. This agent has no inhibitory effects on I_{Kr} at clinical relevant concentrations, and is considered a selective blocker of I_{Na}[26]. It is therefore unlikely that I_{Kr} block contributes to the incomplete antiarrhythmic potential of ranolazine.

The main target for ranolazine, I_{NaL}, is decreased and not increased in CAVB heart. The block of I_{NaL} by ranolazine is insufficient to balance the downregulation of repolarizing K^+ currents (I_{Ks}, I_{Kr}) and ranolazine therefore cannot completely prevent or suppress proarrhythmia. On the other hand, in conditions with increased I_{NaL} (LQT3, heart failure) the block of I_{NaL} should be sufficient as documented in LQT3[12-14] and/or heart failure[15].

Interestingly, in single cells, 15 µmol/L ranolazine fully suppressed EADs, whereas lower concentrations of 5 µmol/L could not. This concentration-dependent effect was also observed by others[8], possibly by enhancing I_{NaL} inhibition by 20%, and induction of additional block of sustained Ca^{2+} currents (~30%). On a background of downregulated I_{NaL}, the higher degree of Na^+ and perhaps Ca^{2+} current inhibition might be required to tip the balance towards strengthened repolarization preventing the development of EADs despite block of I_{Kr}. In the intact animal, the concentration of available ranolazine is likely less and below the critical level for complete suppression of EADs. Higher plasma concentrations as 20 µmol/L would far exceed the therapeutic range (2 to 6 µM), and could experimentally not be achieved in the dog.

Beat-to-beat variability of repolarization

BVR is a reliable parameter to predict drug-induced TdP, and is superior to QT prolongation. A drug that increases BVR is torsadogenic and this is independent of total duration of repolarization; a drug that does not increase BVR is considered safe[23]. Fewer studies have addressed the potential of BVR for predicting successful antiarrhythmic treatment, where one expects a decrease and/or prevention of BVR increase if the drug is antiarrhythmic[38]. The strong antiarrhythmic activity of the Ca^{2+} channel antagonist flunarizine, evidenced by complete inhibition of arrhythmogenic events, is associated with full reversal of BVR to baseline levels and lack of increase upon an arrhythmogenic challenge[39], and is in support of this premise. This finding is unlike the modest effects of Na^+ blockers on BVR, which correspond to the inability of the blockers to fully prevent or suppress EBs and TdP. Proarrhythmic outcome was related to an increase of BVR in the susceptible animal and confirms

the value of BVR as a marker of proarrhythmic risk. BVR was reflected already in ectopic beat formation (Fig 3). In the non-inducible animals there was no change of BVR.

Clinical implications

The observation that ranolazine did not produce any proarrhythmic effects, rather was anti-arrhythmic against dofetilide-induced TdP and EADs, further substantiates that ranolazine is a safe drug despite I_{Kr} blocking properties. The concentration at which maximal anti-arrhythmic effects were seen in the setting of reduced I_{NaL} in the CAVB model (15 µmol/L) was approximately 1.5-3 times higher than the clinical therapeutic concentrations. Yet, anti-arrhythmic efficacy at therapeutic concentrations has been documented in the Merlin Trial[18].

Conclusions

The anti-arrhythmic properties of ranolazine are most obvious under conditions of abnormal and increased I_{NaL}, including LQT3. Albeit with less efficacy, ranolazine and other Na^+ blockers are also antiarrhythmic against EADs and TdP in CAVB dogs with acquired LQT, where I_{NaL} is downregulated due to remodeling. Anti-arrhythmic effects were reflected in BVR, in single myocytes as well as in the intact animal.

Acknowledgements

The study was supported by a grant from the EU FP6 (LSHM-CT-2005-018802, Contica), an unrestricted grant of Cardiovascular Therapeutics (CVT) and by a Veni grant from The Netherlands Organization for Scientific Research (916.56.145) to G. Antoons.

Conflict of interest
None declared

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Chapter 7

Effects of K201 on repolarization and arrhythmogenesis in dofetilide sensitive, anesthetized dogs with complete AV-block

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Submitted

Abstract

Aims: The new anti-arrhythmic drug K201 blocks multiple ion channels and controls intracellular Ca^{2+} release by the ryanodine receptor. The canine model of complete AV block (CAVB) has a decreased repolarization reserve causing an enhanced vulnerability to (drug-induced) Torsade de Pointes (TdP). In this study, the pro- and anti-arrhythmic effects of K201 were investigated at two doses.

Methods: Under complete anesthesia, two doses of K201 (0.1 and 0.3 mg/kg/2 min followed by 0.01 or 0.03mg/kg/min for 30 min) were tested in serial experiments in 10 normal (exp #1) and remodeled (CAVB) animals (exp #2-4). Atrial and ventricular electrophysiological parameters were determined, including beat-to-beat variability of repolarization (BVR). Susceptibility to TdP was assessed with dofetilide (25 µg/kg). K201 was administered after (exp #2) and before dofetilide (exp #3-4).

Results: K201 dose dependently prolonged atrial and ventricular repolarization. Dofetilide caused TdP in 9/10 (90%) animals, which was associated with an increase in BVR from 1.7±0.6 to 3.5±0.8 ms, <0.05*. Neither dose of K201 was able to suppress dofetilide-induced TdP. In contrast to the lower dose of K201 (plasma conc. 300 ng/ml), the higher dose (800 ng/ml) showed a pro-arrhythmic signal in a minority of animals: spontaneous TdP incidence was observed in 1/7 dogs (14%), whereas pacing increased TdP to 3/7 (43%). BVR responded accordingly: no change with the lower (1.0±0.5 to 1.3±0.7) and an increase (1.2±0.4 to 2.9±0.8* ms) with the higher dose of K201.

Conclusions: Class III effects of K201 were found, but only at the higher dose this resulted in pro-arrhythmic events, which was preceded by an increase in BVR. No prevention of dofetilide-induced TdP at either dose of K201 was seen in this sensitive model of TdP.

INTRODUCTION

The novel drug K201 (previously known as JTV-519), a 1,4-benzothiazepine derivative, is known to have anti-arrhythmic and cardio-protective properties against intracellular calcium overload [1], ischemia-reperfusion injury[2,3], heart failure[4], cathecolaminergic polymorphic ventricular arrhythmias (CPVT) and sudden cardiac death[5]. These effects are seen at doses as low as 8 µg/kg/min in various animal models (CPVT mice, guinea pigs, dogs with pacing induced heart failure). In a dose of 30 µg/kg/min, K201 suppressed experimental atrial fibrillation in the dog model of sterile pericarditis[6-8]. Infusions of K201 of 200-400 µg/kg/min have been reported to prolong the QT-interval without inducing TdP and were even able to prevent TdP induction in the methoxamine sensitized rabbit model treated with clofilium[9].

K201's mode of action has previously been explained by its ability to suppress (diastolic) intracellular Ca^{2+} leak from the ryanodine receptor (RyR2) which is the Ca^{2+}-release channel of the sarcoplasmic reticulum (SR)[10]. A possible target, in this regard, is FKBP12.6 / calstabin2 on RyR2, which is stabilized by K201[5,11]. However, this compound likely has more complex mechanisms of action because potentially important additional targets of action have been described including: block of I_{Na} (IC_{50}=1.2-2 µM), I_{CaL} (IC_{50}=3 µM), I_{K1} (IC_{50}=5 µM) and I_{Kr} (IC_{50}=1.2 µM) in guinea pig ventricular myocytes[12], $I_{K,Ach}$ (IC_{50}=0.12 µM) and I_{Kr} antagonism in guinea pig atrial cells[6], α1-adrenergic block in rat myocytes [1] and $I_{K,ATP}$ opening properties in guinea-pig ventricular muscles[3]. These actions on de- and re-polarizing ion currents may affect the duration of the action potential (APD) differently depending on ion channel distribution and their relative contribution. Atrial APD lengthening[6] and ventricular APD shortening[13] have been described in guinea pigs. In contrast, there are reports of a prolonged QT-duration in rabbits and dogs humans[7,9]. Whereas lengthening of the atrial repolarization has been linked with therapeutic effects in atrial fibrillation and flutter, lengthening of ventricular repolarization has been associated with both anti- as well as pro-arrhythmic effects. Because the TdP rabbit model relies on α-adrenergic stimulation (methoxamine), which is a target of K201[1,9], it is possible that the anti-arrhythmic effect of this drug is based upon this mode of action. The tendency of this drug to produce or prevent TdP in other animal models with no or less reliance on α-adrenergic stimulation is unknown.

The rationale of this study was to examine the effects of K201 on ventricular repolarization and on the occurrence of TdP. K201 was given at two doses to anesthetized dogs with normal (sinus rhythm) or remodeled hearts due to chronic, complete AV-block (CAVB). This model has an enhanced susceptibility for drug induced TdP and offers the opportunity to serially study the anti- as well as pro-arrhythmic properties of K201 using TdP as an end-point. The analysis included measurements of beat-to-beat variability of repolarization (BVR)[14,15], a parameter suggested to have more predictive power for the detection of pro-arrhythmic signals as QT-time.

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

Anesthesia and general experimental protocol

Ten adult mongrel purpose bred dogs (Marshall, USA; body weight: 23±2 kg, 4 females) were included. Experiments were performed under complete anesthesia after overnight fasting. Premedication consisted of 0.5 mg/kg methadone, 0.5 mg/kg acepromazine and 0.5 mg atropine i.m. Anesthesia was induced with nembutal (pentobarbital 25mg/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 a thermal mattress to maintain body temperature, fluid administration to prevent volume depletion (0.5 L 0.9% NaCl), and administration of antibiotics and analgesics. In between experiments, at least 2 weeks expired to allow full recovery of the animals.

Standard 6-leads ECGs with 4 precordial registrations and endocardial monophasic action potential (MAP) recordings (Hugo Sachs Electronics, Germany) were gathered from the left and right ventricular (LV and RV) wall. The latter catheter was temporarily replaced to record signals from the right atrium (RA) as well (figure 1).

Experimental protocol

The 4 serial experiments are illustrated in figure 1: in exp# 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 (SR: unremodeled heart). Only after the higher dose, LV pressure was determined using a 6F pressure catheter (Sentron, Roden, Netherlands). At the end of this experiment, irreversible AV block was induced by radiofrequency ablation as previously described[16].

After at least 3 weeks of AV block (exp #2), the dogs with remodeled hearts[17] were challenged with dofetilide (0.025 mg/kg in 5 min i.v.), a specific I_{Kr} blocker, to determine their vulnerability for TdP (figure 1). In general, dofetilide induces repetitive TdP in about 75-80% of the dogs with CAVB[16,18]. Dofetilide infusion was stopped when TdP occurred and the exact administered dose was recorded. When repeated TdP ($\geq$3) was seen, the anti-arrhythmic effects of the two doses of K201 were investigated.

The last two experiments (figure 1) were performed to determine the pro-arrhythmic effects of K201 infusion. In addition (exp #3-4), in a random crossover design, the anti-arrhythmic potential of K201 to prevent dofetilide-induced TdP was assessed by re-administering the arrhythmogenic dose of dofetilide. Besides the regular electrophysiological parameters, RV atrial and ventricular effective refractory period (RA ERP and RV ERP) were determined (protocol of electrical stimulation, PES, hatched bars) at baseline and after K201 administration (figure 1). After 2 min of steady state pacing, RV ERP was determined with pacing from the RV MAP catheter using a train of 8 paced beats with 800 ms cycle length

(CL) and with a pacing output of two times the diastolic threshold followed by an extra stimulus using a decremental design (starting from a CL of 300 ms) in steps of 5 ms till the ERP was reached. RA ERP was determined with pacing from the RA MAP catheter at 250 and 400 ms drive CL's with a pacing output 4 times the diastolic threshold.

K201 (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/2 min i.v. followed by 0.01 or 0.03 mg/kg/min respectively for 30 min. Plasma concentrations of K201 were determined at regular time points.

Data analysis

Signal processing, data recording and amplification were done as previously described [19]. RR and QT interval in lead II, LV and RV MAP duration (LV and RV MAPD) at 90% repolarization and RA MAPD at 50 % repolarization were measured off-line using a custom-made computer program (ECG-Auto, EMKA Technologies, France) at various time points. QT intervals were corrected for heart rate (QTc) with van de Water method[20]. The interventricular dispersion of repolarization (Δ MAPD) was calculated as the difference between the LV MAPD and RV MAPD. Data measurements were averaged from 5 consecutive beats. Beat-to-beat variability of repolarization duration was quantified with short-term variability (STV) from LV MAPD using 31 consecutive beats: $STV_{LV}= \sum|LV\ MAPD_{n} - LV\ MAPD_{n-1}| /30*\sqrt{2}$. STV_{LV}[21]. In case of pro-arrhythmic events, STV measurements were made in proarrhythmic circumstances immediately prior to the first drug-induced extra systole and if possible at fixed time points.

Quantification of arrhythmias

Distinction between single (SEB) or multiple (MEB) ectopic beats, defined as spontaneous beats initiating before the end of the preceding T wave, was made as the latter are considered more pro-arrhythmic[22]. TdP was defined as a polymorphic ventricular tachyarrhythmia with a twisting shape (variable axis) of at least 5 consecutive beats. A dog was considered to be TdP inducible when this characteristic tachyarrhythmia occurred at least 3 times. If TdP did not stop within 10 sec or when the arrhythmia deteriorated into VF, electrical cardioversion was performed via thoracic patches placed in advance. The incidence and duration of TdP were quantified (figure 1) over a 10 min period after the start of dofetilide administration (black bar) and compared to 10 minute intervals at baseline and after K201 administration.

Statistical analysis

Pooled data are expressed as mean ± standard deviation (sd). Comparisons were performed with 1-way repeated-measures ANOVA. A 2-way repeated measures ANOVA compared the effects of both doses K201 on electrophysiological parameters (experiments 3 and 4) with a Bonferroni correction. For two groups a paired t-test was applied. For non-parametric comparison, a Fisher's exact or Kruskal-Wallis test was used followed by Dunn's test. Statistical significance was defined by $P<0.05$.

Results

Because the plasma concentrations did not differ between SR and CAVB dogs, we present them together in figure 2, upper panel. Steady state plasma concentrations between 10-30 min after the start of K201 (figure 2, upper panel) were 300 (n=8) and between 700-900 ng/ml (n=9), respectively. Immediately after the bolus (relevant for exp# 2), the plasma concentrations were higher.

Electrophysiological effects of K201 in anesthetized dogs in normally conducted sinus rhythm (SR).

Both dosages of K201 increased repolarization parameters (table 1). With the lower dose, these increases were almost exclusively seen at 30 minutes (table 1, upper panel) while with the higher dose lengthening of repolarization was already evident 15 minutes after the start of the infusion (table 1 and figure 2, lower panel). There was no dose dependent effect, with the exception of RA MAP duration which was prolonged only after the higher dose. In the higher dose, electrophysiological effects remained present for at least 15 min (table 1 and figure 2, t=45 min) after stopping the infusion, whereas there was a quick reduction to intermediate values with the lower dose of K201. The higher dose was free of negative inotropic effects. LV dP/dt max was not changed: 1340±193 mmHg/s in control to 1333±299 mmHg/s, after 30 min of K201 infusion.

K201 effects on dofetilide-induced Torsade de Pointes.

In dogs with CAVB, dofetilide administration (n=10) resulted in a significant increase in 1) RR interval, 2) most repolarization parameters, and 3) STV_{LV} (from 1.7±0.6 to 3.5±0.8 ms). Moreover, dofetilide caused reproducible TdP in 9 of 10 canines. Arrhythmia quantification in this group (n=9) revealed 10±7 episodes of TdP, 20±26 MEBs and 52±58 SEBs during the 10 min observation period. Average TdP duration was 9.5±5 sec and 4±4 episodes TdP needed to be electrically defibrillated. Of the 9 dogs with spontaneous TdP after dofetilide, one animal did not receive K201 in the second part of the experiment, because a defect in the defibrillation patches required immediate anti-arrhythmic intervention. For that purpose, we used a fast working, established anti-dote, levcromakalim[15]. In the remaining 8 individuals, five dogs received the higher and 3 animals the lower dose of K201. No reduction in TdP incidence was achieved with K201, a finding independent of dose. Both the number (nr) of TdP (from 9±8 to11±11 after the higher dose or from 13±9 to 16±4 with the lower dose of K201) and their severity expressed as number of defibrillations were unchanged after adding K201. However, the duration of TdP was significantly shortened (from 9.3±6.3 to 4.4±2.6 s after higher dose K201, or 13±9 to 6±2 s after the lower dose). This reduction in duration was accompanied by a significant increase in the occurrence of SEBs (from 52±58 to 130±95, <0/05) and MEBs (from 20±26 to 43±25 beats, <0.05).

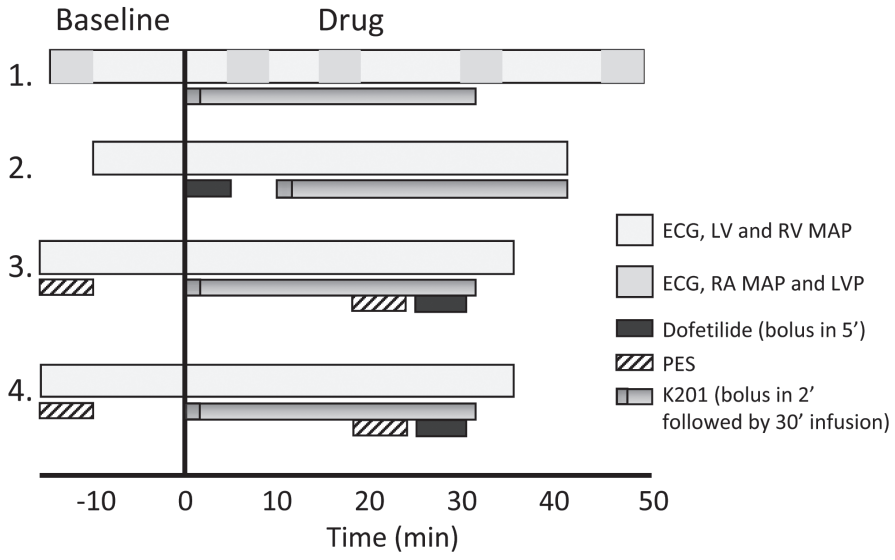

Figure 1: Flow chart of the serial experiments performed in this study.
Each dog was planned to undergo 4 experiments. In exp #1, prior to AV-block induction, the electrophysiological effects of the lower (n=5) and the higher dose (n=5) of K201 were tested in normal hearts. Atrial and ventricular repolarization and left ventricular pressure parameters were measured. In exp #2, susceptibility testing with dofetilide was performed 3 weeks later (10 dogs with CAVB). When inducible, K201 was given in the second part of the experiment to test anti-arrhythmic potential of the higher and lower doses. In exp #3-4, the 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 this experiment dofetilide was administered to test the anti-arrhythmic preventive potential of K201.

Electrophysiological effects of K201 in anesthetized dogs with CAVB

One dog was lost at the end of experiment 2, leaving 8 dogs for the remainder of the protocol (exp# 3 and 4), which included (figure 1) pacing trains to determine ERP. One dog was excluded due to pacing induced TdPs at baseline, leaving 7 animals for the serial tests of arrhythmia inducibility with programmed stimulation after K201 administration. In table 2 and figure 3, the effects of dofetilide and K201 on serially tested dogs are presented (n=7).

a) Lower dose of K201: 0.1 mg/kg/2' + 0.01 mg/kg/30'

The lower dose of K201 caused lengthening of most repolarization parameters, with the exception of RV ERP and STV_{LV} (table 2 (middle column) and figure 4). No spontaneous TdP

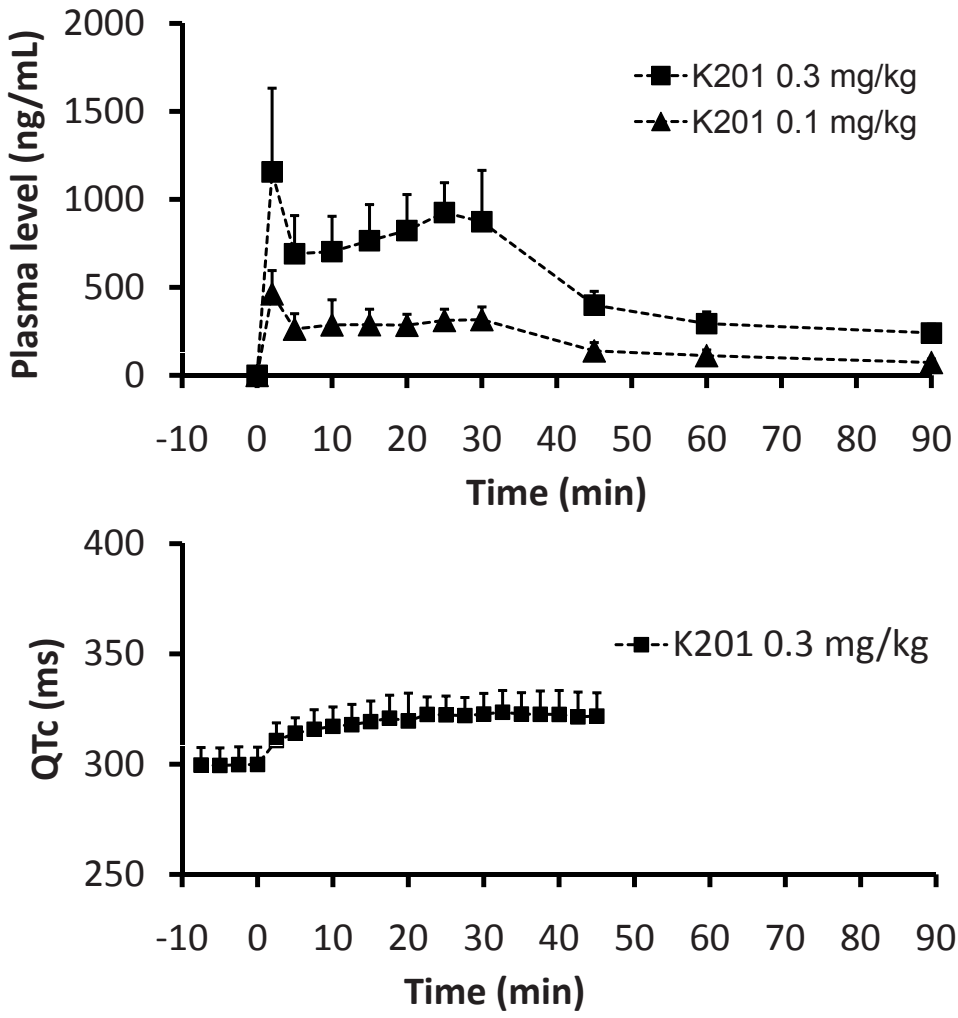

Figure 2: Plasma concentrations of K201 with its effect on QTc.
Upper panel: time dependent plasma concentrations of K201 after bolus (2 min) and maintenance infusion (30 min) of the lower and higher doses. Please note that the maintenance infusion prevented rapid decline in K201 concentrations (upper panel) thereby creating a steady state effect. Lower panel: time dependent effects of higher dose K201 on QTc. An increase in QTc was seen (class III effect), which became significant from 2.5 min.

Table 1: Electrophysiological effects of two doses K201 in anesthetized dogs in normally conducted sinus rhythm (exp. # 1)

Dose: 0.1 mg/kg (n=5)	Baseline	K201 15'	K201 30'	K201 45'
RR	581±37	598±38	605±35 *	614±42 *
QT	280±18	300±8	306±7 *	293±11
QT_c	316±16	335±8	340±8 *	327±9
LV MAPD	213±19	224±10	240±17 *	234±20
RV MAPD	197±5	211±11 *	210±10 *	
∆MAPD	16±15	13±12	29±14	
$RAMAPD_{50}$	82±30	84±16	83±20	
Dose: 0.3 mg/kg (n=5)				
RR	589±16	625±30 *	618±42	630±51 *
QT	261±10	284±10 *	285±11 *	291±13 *
QT_c	297±10	317±9 *	318±10 *	323±11 *
LVMAPD	209±21	226±24	240±20 *	247±11 *
RVMAPD	190±15	220±9 *	218±16 *	224±18 *
∆MAPD	19±32	6±18	22±16	23±13
$RAMAPD_{50}$	101±11	106±1	125±13 *	

All values are in ms, * P<0.05 versus baseline

Table 2: Electrophysiological effects of dofetilide, lower and higher doses of K201 in serial experiments in anesthetized dogs with CAVB (n=7, exp.# 2-4)

	Baseline 2	Dofetilide	Baseline 3	K201 0.1 mg/kg	Baseline 4	K201 0.3 mg/kg
RR	1129±179	1316±260*	1144±160	1206±158*	1218±172	1371±215*
QT	432±77	591±116*	413±54	460±76*	429±63	528±72*#
QT_c	421±68	564±98*	400±50	440±70*	410±61	496±55*#
LV MAPD	333±59	483±103*	309±44	387±62*	338±51	458±78*#
RV MAPD	287±40	387±84*	273±25	313±28*	290±22	360±54*
∆MAPD	46±24	96±100	36±29	74±49*	48±40	98±61*
STV_{LV}	1.5±0.5	3.1±0.5*	1.0±0.5	1.3±0.7	1.2±0.4	2.9±0.8*#
RV ERP			247±25	253±14	248±26	293±28*#
RA ERP			123±9	151±19*	112±18	141±17*

All values are in ms, * P<0.05 versus baseline

were seen with this dose. Only single ectopic beats occurred in a single dog (figure 3, middle column). With PES, no arrhythmias were induced at baseline nor after K201 infusion.

b) Higher dose of K201: 0.3 mg/kg/2' + 0.03 mg/kg/min

A dose dependent lengthening of repolarization parameters in the CAVB group was observed for $QT_{(c)}$, LV MAPD, STV_{LV} and RV ERP (table 2, right column and figure 4). Between 10-20 min after the start of the higher dose of K201 (second observation period, STV_{LV} significantly increased and multiple self terminating TdP (n TdP= 9) appeared in one animal (figure 5) with an average duration of 2.5±1 s. In addition, PES resulted in the induction of TdP in 2 other animals.

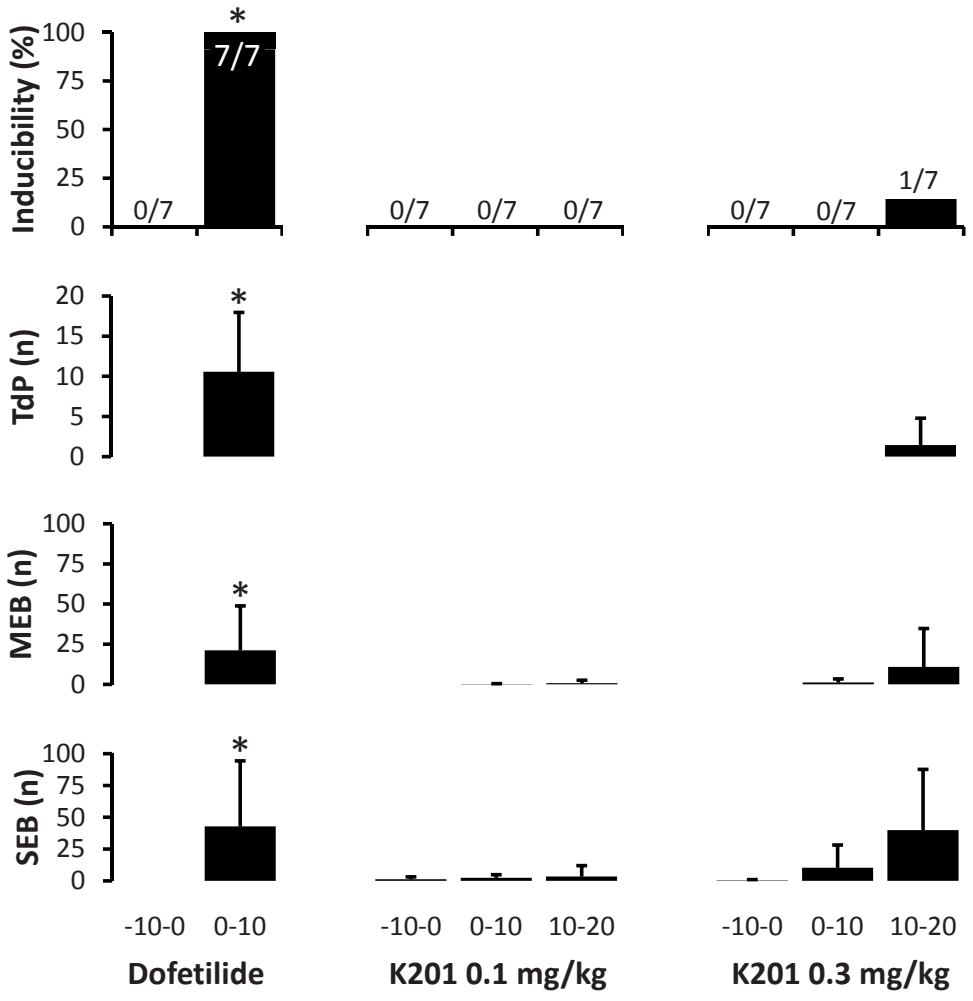

Figure 3: Pro-arrhythmic effects of dofetilide (left), lower or higher doses of K201 in serial experiments (exp #3-4) in dogs with CAVB (n=7).

Quantification of pro-arrhythmia was performed by: TdP inducibility as the relative number of dogs showing at least 3 TdPs (top), average number (n) of TdP, mean number of multiple ectopic beats (MEB) and single ectopic beats (SEB, lowest panel). Bars represent the mean ± sd per 10 min time interval. * <0.05 vs. the 10 min control period preceding drug infusion.

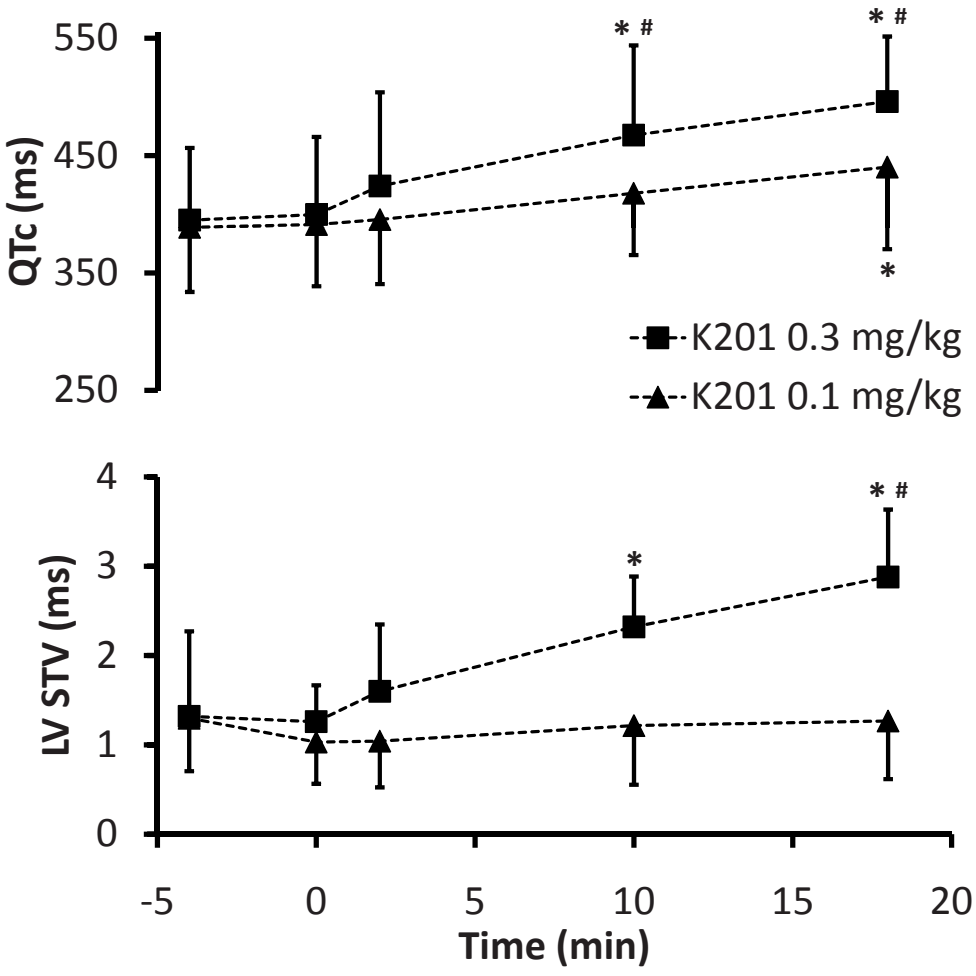

Figure 4: Dose dependent electrophysiological effects of two dosages K201 on QTc and STV in dogs with CAVB

The higher dose increased both QTc (upper panel) and STV (lower panel), whereas the lower dose only increased QTc. These effects were dose-dependent:

* represents: < 0.05 vs. baseline and #, higher vs lower dose of K201.

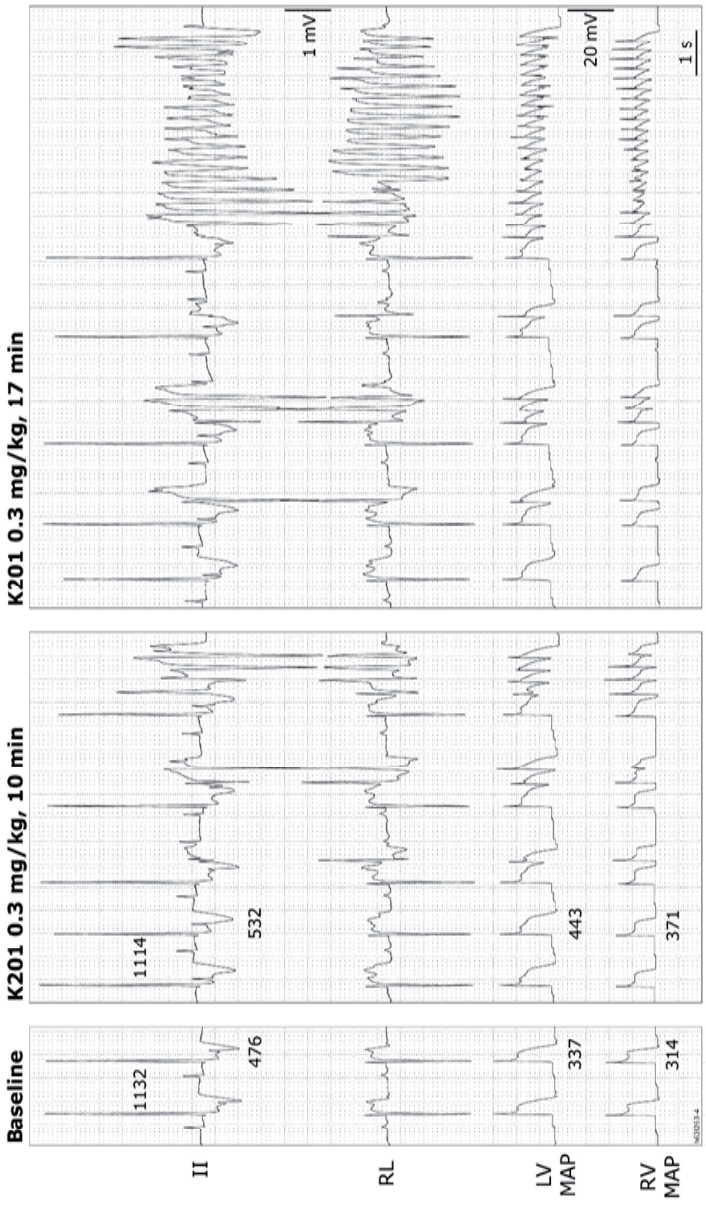

Figure 5 Individual example of TdP induction with the higher dose of K201
Two ECG leads (II and RL) and 2 endocardially placed MAP recordings are shown at 10 mm/sec on scale paper. Three panels illustrate baseline (left), and 2 time points (10 and 17 min) after the higher dose K201. At 10 min (middle panel), the first pro-arrhythmic activity in the form of single and multiple ectopic beats were observed. Please note that the ectopic activity arises from within the MAPD.
Later in time, TdP appeared (right panel). The numbers depict RR and QT intervals and the duration of the LV and RV MAP.

Anti-arrhythmic action of K201 in preventing dofetilide induced TdP

a) Lower dose of K201: 0.1 mg/kg/2' + 0.01 mg/kg/30'
There was no preventive action of this dose of K201: dofetilide administration still initiated spontaneous TdP in 6/7 dogs.

b) Higher dose of K201: 0.3 mg/kg/2' + 0.03 mg/kg/min
Following PES, TdP reappeared and hindered further investigations. There-fore, only in 4/7 dogs the preventive protocol of the higher dose K201 against dofetilide-induced pro-arrhythmia could be completed. No preventive anti-arrhythmic effects were noted.

Discussion

Our results can be summarized as follows: In the CAVB dog, K201 1) prolongs atrial and ventricular repolarization dose-dependently, 2) has no significant anti-arrhythmic effects against dofetilide induced TdP, and 3) enhanced the pro-arrhythmic effects of programmed stimulation at the higher of the two doses examined.

1. Prolongation of repolarization

The duration of the cardiac action potential is the result of numerous in- and outward currents / pumps that operate as a team. The measured steady state plasma concentrations of K201 are in line with those reported in other studies: 300 ng/ml after the lower dose[23,24] and 700-900 ng/ml with the higher dose[7]. These values translate into 0.7 μM and between 1.5-2 μM, respectively (molecular weight of K201 is 461), which are close to the IC_{50}s of many currents (see introduction) without considering protein binding. Due to many effects of K201 on ion channels and receptors, it is difficult to predict the effect of the drug on repolarization times.

Both in the control as in the dog with CAVB, K201 showed class III effects. Although these effects occurred earlier and persisted longer with the higher dose, there was no dose-dependent finding in normal hearts with the exception of RA MAPD (table 1). In remodeled hearts, dose dependency of K201 was seen in most electrophysiological parameters (figure 2). Prolongation of atrial and ventricular repolarization has been described by others too using the higher of the two doses: AERP and QTc increases in SR dogs[7] and for QTc in rabbits[9]. Differential effects on atrial and ventricular repolarization times, as suggested from studies in guinea pigs[6,13] were not observed in this study.

2. No relevant anti-arrhythmic effects against dofetilide-induced TdP

Intracellular calcium handling is a complex, fundamental process for the proper function of cardiac myocytes responsible for excitation-contraction coupling. The rapid increase in free cytoplasmic Ca^{2+} through the L-type Ca^{2+} channel triggers a more abundant Ca^{2+} release

from the SR via RyR2. Ca^{2+} reuptake in the SR takes place by a Ca^{2+} pump (SERCA), whereas Ca^{2+} extrusion from the cell is provided by a number of pumps, mainly through Na^{+}/Ca^{2+} exchanger (NCX). Dysfunction of the calcium handling in various pathophysiological conditions is linked to cardiac arrhythmias, including inherited CPVT and heart failure[4,25-30]. Triggering of these arrhythmias may lie in a diastolic SR calcium leak that can activate the transient inward current of the NCX, generating delayed afterdepolarizations (DAD) and possibly (runs of) ventricular triggered beats. K201 has been shown to provide stabilization of FKBP12.6, thereby preventing diastolic SR Ca leak and associated VTs[5].

A second triggered arrhythmic mechanism is the initiation of early afterdepolarizations (EADs). A prolonged APD with problems in Ca^{2+} handling may provide a second depolarization within the AP, most likely through window currents. In this canine CAVB model, both DADs as EADs are well documented[31-34]. In case of drug induced TdP, EADs as the initiating mechanism seem to prevail. The anti-arrhythmic properties of K201 against TdP are therefore of interest mechanistically.

Recently, quantification of dofetilide-induced TdP has been performed in a number of ways and we showed that TdP induction was repeatable and reproducible over weeks. Furthermore dofetilide-induced pro-arrhythmic response lasts for more than 20 minutes allowing repeated anti-arrhythmic drug testing[17]. Thus the suppressive properties of K201 could be elucidated between 10-20 minutes after dofetilide (exp #2), and secondly its preventive potential against dofetilide-induced TdP could be investigated (exp #3-4). Independent of dosage, K201 was not able to suppress or prevent TdP in this model.

This lack of anti-arrhythmic effect is in contrast with the results obtained in the α- adrenoreceptor 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 TdP[9]. As mentioned, a confounding variable in this rabbit model is the α- adrenoreceptor antagonist effect of K201[1]. The lack of anti-arrhythmic effect may argue against a primary involvement of the RyR–calstabin complex in the generation of ectopic beats and the induction of drug induced TdP in this model. Caution is however needed, because a) this protective mechanism may be counteracted by the repolarization delay, K201 has a combined I_{Kr} and I_{K1} block[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 recently been demonstrated in knock-in RyR4496 mice that were not protected by K201 (1 and 10 μM) against isoproterenol induced DADs and VT[24]. The lack of antiarrhythmic effects was also surprising as the block of $I_{Ca,L}$ and late I_{Na} is known to be antiarrhythmic in this model[31,35]. The observation in the suppression experiments that TdP duration shortened while the occurrence of SEBs and MEBs intensified may also be explained by the many mode of actions of K201.

3. Pro-arrhythmic signals with of K201

Because K201 was administered before dofetilide in the first part of exp #3- 4, its pro-arrhythmogenic potential could be investigated. The anesthetized dog with CAVB is very sensitive to drug induced TdP[17]. Whereas, the clinical TdP incidence after class III drugs varies

from 2-5%[36], these drugs cause TdP in 70-80% of CAVB dogs[18,37]. On the other hand, the model also shows specificity as evidenced by the fact that a number of drugs do not induce TdP despite (severe) prolongation of QT duration (figure 6). Recently, it was proposed that STV_{LV} could predict pro-arrhythmic properties of drugs by demonstrating a sudden increase prior to TdP, whereas drugs less likely to cause TdP did not change STV_{LV}. In the past, specific pacing modes were used to increase TdP incidence in this model. An example is d-sotalol (2 mg/kg) that had a low TdP incidence in the absence of pacing (5-25%), but with PES this value increases to approximately 50% of the animals[38]. A similar observation was made with chronic dronedarone treatment: TdP occurrence increased from 3/8 to 4/8 of the dogs[39], as with almokalant from 9/14 to 12/14 animals[37] (figure 6).

In this study, dofetilide induced TdP in 9/10 animals, which is at the higher extreme of the results of previous studies[17] with this Ikr blocker (figure 6). The enhanced susceptibility of the dog with CAVB can be explained by a reduced repolarization reserve and disturbed [Ca]i handling[14].

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)[7,9]. With the lower dose, this assumption was confirmed. However, the higher dose of K201 revealed several signs of pro-arrhythmia: 1) reproducible TdP induction in one animal (1/7 = 14%), 2) pacing induced TdP in 2 more animals, and 3) a significant increase in STV_{LV} (table 2). When comparing the higher dose of K201 to dofetilide, it is evident that QT-prolongation is less (table 2) and that TdP incidence is much lower (1/7 versus 9/10 or 7/7). Still, the TdP incidence of the higher dose of K201 resembles the results obtained with d-sotalol (2mg/kg). The fact that pacing increases the number of animals with TdP is also in line with previous results. However, the results of the pro-arrhythmic screening in this model differ between group comparisons: for dofetilide, TdP incidences varied between 70% and 90% (in this study). And because d-sotalol, dronedarone and almokalant were never serially compared to dofetilide, we cannot rule out that these inter-individual differences can be responsible for an overestimation of TdP after the higher dose of K201. This group of animals was very sensitive to induction of TdP.

4. Clinical implications

Both doses of K201 increased the atrial ERP which could confer an anti-arrhythmic effect for atrial fibrillation or other arrhythmias. Further, the effects of K201 on intracellular Ca^{2+} cycling could have novel anti-arrhythmic effects in atrial fibrillation[8] as well as in ventricular tachycardias[5]. K201 is currently being tested in humans with atrial fibrillation. Most antiarrhythmic drugs currently used to treat clinical arrhythmias that delay cardiac repolarization have the capability of producing TdP clinically, and in the CAVB model. The findings in this study suggest that K201 prolongs the QT but has a lesser tendency to produce spontaneous TdP in the doses studied than does dofetilide (figure 6). Nonetheless, K201 enhanced the ability of PES to produce TdP in this model and resulted in spontaneous TdP in one animal suggesting that there may be a potential for K201 to produce clinical TdP. Further studies will be required to reveal the anti- and pro-arrhythmic effects of this agent in other model

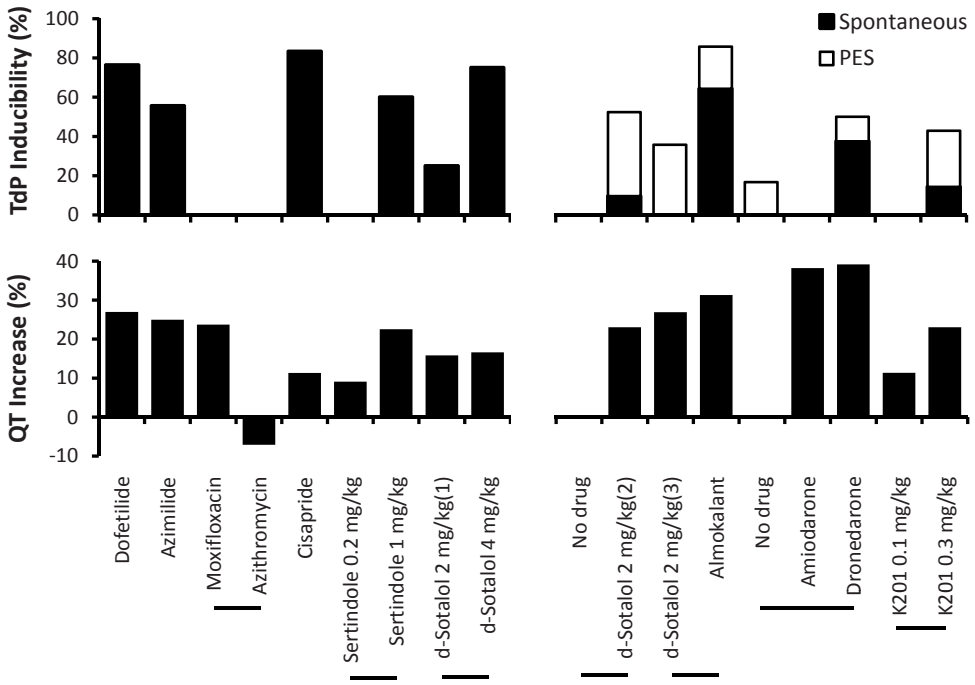

Figure 6 Overview of a number of drugs that have been evaluated for pro-arrhythmic properties in the anesthetized CAVB dog model.

Upper panel: spontaneous inducibility of TdP (≥ 3 times) after drug administration (black bars) ranges from 0 to 80% depending on the drug and the administered dose. On the right side, the contribution of programmed electrical stimulation (PES) for TdP inducibility is shown (white bars). The rate changes induced by PES enhance the susceptibility to (drug-induced) TdP considerably.

Lower panel: the effects of these drugs on QT parameter are presented as the relative increase of the mean. From left to right, the number of dogs and the reference of the study are: dofetilide [17] (n=72), azimilide [18] (n=9), moxifloxacin (n=6) and azithromycin [40] (n=6), cisapride [41] (n=6), sertindole [42] low (n=5) and high dose (n=5), d-sotalol [21] low (1) (n=8) and high (n=8); no drug (baseline) and d-sotalol low [38] (2) (n=18); d-sotalol [37] low (3) and almokalant [37] (n=14, in serial experiments); no drug (n=6), amiodarone (n=7) and dronedarone [39] (n=8); K201 low (n=7) and high dose (n=7) (data from present study). All drugs were administered iv., except amiodarone and dronedarone, which were administered chronically per os.

systems, and in man, where affinity for various receptors and protein binding of K201 could be different. The results of this study, in which a 3-fold difference in plasma concentrations of K201 showed different arrhythmic outcome, must be considered when studying K201 in certain patient populations at risk for pro-arrhythmia.

Conclusions

Dose dependent class III effects of K201 were documented, with evidence for modest pro-arrhythmic potential, linked to an increase in BVR, at the higher dose. No relevant anti-arrhythmic effects against drug-induced TdP were seen in susceptible dogs with CAVB.

Acknowledgements:

Drs. Oros was funded by a grant of the European Union: CONTICA (EUFP6-STREP-LSHM-CT-2005-018802). Disclosure: this study was co-financed by Sequel Pharmaceuticals Inc (San Diego, Ca, USA).

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Chapter 8

General discussions

Excitation-contraction coupling

Proper function of the heart starts at the sino-atrial node. The pacemaker cells form the electrical impulse that propagates through the conduction system to activate the atria and ventricles. At the cellular level, depolarization leads to an action potential (AP), which starts intracellular cycling of calcium to enable cell shortening (figure 1). To overcome depolarizing currents and to end the AP, repolarization is enabled, mainly through four K^+ channels: the transient outward current (I_{to}) for the fast repolarization (phase 1), the rapid and slow component of the delayed rectifier (I_{Kr} and I_{Ks}) to overcome the plateau phase (phase 2) and the inward rectifying current (I_{K1}) for maintaining the diastolic resting membrane potential. When the electric impulse reaches a myocyte, the (inward) Na^+ current (I_{Na}) is activated, depolarizing the cell and inducing the calcium current (I_{Ca}). Calcium that enters the cytoplasma activates Ca^{2+} release from the sarcoplasmic reticulum (SR) through the ryanodine receptor (RyR2) which is followed by contraction. The intracellular Ca^{2+} concentration is restored by re-uptake into the SR through sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2) and by transport to the intercellular space mainly by Na^+-Ca^{2+} exchange (NCX). Intracellular Na^+ concentration is the main regulator of NCX and is also maintained through several pathways. For a detailed overview of this complex mechanisms of de- and repolarization processes related calcium handling, we refer to two published reviews[1,2].

As repolarization and calcium cycling (figure 1) involve multiple pathways, the mechanisms to accomplish a normal electrophysiological and mechanical function are essentially protective and are the basis for the cardiac functional reserve. Only multiple adaptations, disorders or challenges can disturb the normal physiology and lead to rhythm disorders and pump dysfunction.

Arrhythmias are often classified according to their mechanisms: abnormal automaticity, reentry (abnormal conduction) and triggered arrhythmias but in reality they often are a combination of these mechanisms. One such example is atrium fibrillation where both reentry and triggered mechanism are involved[3,4].

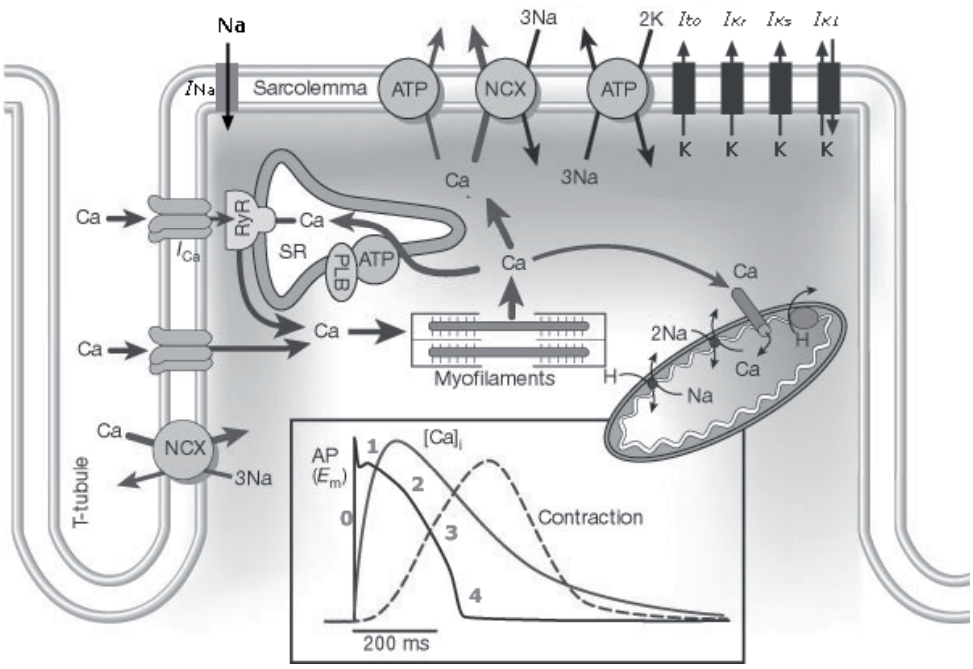

Figure 1 Excitation-contraction coupling and repolarization process
This figure describes schematically the depolarization-repolarization of the action potential (inset) and excitation-contraction coupling: I_{Na} depolarizes the cell and activates I_{Ca} which induces calcium release from the sarcoplasmic reticulum (SR) through Ryanodine Receptor (RyR) followed by contraction. Repolarization is dominated by I_K which restores the resting membrane potential preparing the cell for a new cycle. Relaxation is followed by Ca^{2+} reuptake into SR and extrusion out of the cell through the sodium-calcium exchanger (NCX). Inset shows the dynamics of action potential, Ca^{2+} transient and contraction measured in a rabbit ventricular myocyte. Adapted from Bers 2002 [2]

Torsades de Pointes arrhythmias (TdP)

Although TdP is often described as an adverse reaction to various pharmaceutical compounds with class-III effects (I_{Kr} blockers), this polymorphic ventricular tachycardia was initially described in the absence of drugs in a woman in which acute, total Atrio-Ventricular (AV) block caused severe bradycardia and altered ventricular activation[5]. Multiple factors (hits) disturbing the repolarization process can predispose the heart and elicit TdP arrhythmias.

The electrophysiological mechanisms underlying TdP are important to be completely understood as the successful prevention or treatment depends on the right mechanistic approach. TdP mechanisms are complex and therefore still under debate. They are definitely

related to a disturbed and prolonged repolarization. There is evidence for a dominant role for focal activity in the initiation of TdP[6-8]. Whether this focal activity is also important for perpetuation is still investigated. Alternatively, reentrant mechanisms could be relevant for perpetuation too as TdP may degenerate into VF, an arrhythmia based upon this mechanism[6-9]. As cellular explanations for the focal source, early (EADs) and delayed afterdepolarizations (DADs) have been well documented in the initiation of triggered arrhythmias[9-13]. EADs are most likely generated by window currents (most likely I_{Ca}), although involvement of the NCX mediated inward current cannot be ruled out[12,14-16]. DADs are thought to be mainly due to diastolic calcium leak from the SR[17-19] that occurs in intracellular calcium overload. The unconditional release of calcium activates an NCX-dependent inward current. However, there is also evidence that their etiology may be similar because EADs and DADs can occur in the same preparation [16, 18, 20] and calcium leak from the SR has been shown to initiate EADs[16,18,20,21].

Antiarrhythmic targets: sarcolemmal and intracellular targets

Cardiac adaptations and ion channel remodeling in the dog with chronic, complete AV-block (CAVB) are in detail reviewed in chapter 2. In short, the prolongation of repolarization and the reduced repolarization reserve can be attributed to 1) a reduction in I_{Kr} and I_{Ks} and an increase in both modes of I_{NCX} function, and to 2) a disturbed Ca^{2+} homeostasis related to an increased SR Ca^{2+}concentration, I_{NCX} and $[Na^+]i$ sub-sarcolemmal. Thus, the dog with CAVB is predisposed for repolarization dependent arrhythmias (chapter 3). Any additional, acute disturbance in the repolarization and/or calcium cycling can be the final hit that leads to TdP. In consequence, based upon this background, several anti-arrhythmic targets can be proposed: block of I_{Ca}, late I_{Na}, I_{NCX}, stabilization of RyR2 with its regulating unit calstabin2 (FKBP12.6), a reduced SERCA2 with its regulatory unit phospholamban and block of the Ca-Calmodulin dependent kinase II (CaMKII) with its multiple intracellular facilitating functions on ion channels and components of the calcium cycling. Alternatively, shortening of repolarization can be achieved by potassium channel openers, isoproterenol or pacing. In this thesis, a number of these targets have been examined using pharmacological tools.

In chapter 4, we start with AVE0118, a drug developed against atrial fibrillation. Because this drug blocks I_{to}, the plateau phase may be at a higher voltage thereby allowing the voltage dependent delayed rectifier current to operate stronger. This may lead to a shortening of ventricular repolarization, which may be anti-arrhythmic against TdP. However, no such action was observed.

Flunarizine and verapamil (chapter 5) were chosen for their calcium antagonism, specifically for blocking L-type calcium channel (I_{CaL}). They are both known to block EADs and DADs and have been shown to be antiarrhythmic against TdP. However, they are not very specific as they have additional blocking effects on other ion channels too: e.g. a strong blocking effect against I_{Kr} which normally is considered proarrhythmic. It was shown that, at the doses studied, flunarizine was highly effective by combining block of I_{Ca} with that of late I_{Na}, whereas verapamil achieved its strong anti-arrhythmic effects against dofetilide induced TdP by combining I_{Ca} block with a possible reduction in Ca^{2+} sparks (table 1).

Table 1: Ion-channel targets of the studied drugs, divided in possible pro- and anti-arrhythmic actions

Drug	Plasma levels (for used dosages)	'Pro-arrhythmic'		'Anti-arrhythmic'		
		I_{Kr}	*Other*	$I_{Ca,L}$	I_{Na}	other
Ave0118 [39-42]	3.9 μM (3 mg/kg/h)	10 μM				
Flunarizine [43-47]	≈ 1.7 μM (2mg/kg/2')	5.7nM	I_{Ks}: 0.76 μM	4.6-10 μM	1 μM: 95% late I_{Na} 10 μM: 10% peak I_{Na}	
Verapamil [45, 47-52]	≈ 0.5 μM (0.4 mg/kg/3')	0.1-3 μM	I_{Ks} 6 μM	0.6-15 μM	-	10 μM: ↓ 35 % Ca^{2+} sparks
Ranolazine [53-57]	15-20 μM (0.225 mg/kg/min)	12 μM	-	50 μM	late $I_{Na,}$ 6 μM	
Lidocaine [58-60]	≈ 12 μM (3 mg/kg/3')	-	-	-	3-5 μM	
K201 [61-63]	0.7 μM (0.01 mg/kg/min)	1.2 μM	I_{K1}: 5 μM	3 μM	1.2-2 μM	FKBP12.6 stabilizer (0.3-1μM)

The IC_{50} concentrations of the drugs in order to block the ion current are provided

As Na^+ current blockers, ranolazine and lidocaine were studied (chapter 6). The advantage of lidocaine is its specificity for blocking the I_{Na}, whereas ranolazine has additional blocking effects, such as blocking I_{Kr}. There was no difference in the anti-arrhythmic properties of these two drugs K201 was selected (chapter 7) for its proposed stabilizing effect on RyR2 leakage induced Ca^{2+} sparks. Mechanistic the use of this novel compound is limited because it lacks specificity (see table 1).

Anti-arrhythmic efficacy

To compare the results of the different anti-arrhythmic drugs used, tables 2 and 3 were prepared. In all experiments these drugs were administered to susceptible dogs that had received dofetilide as the pro-arrhythmic medication. TdP inducibility was defined as more than 3x TdP per individual in the 10 min post-dofetilide period. Repeatability and persistence of dofetilide-induced TdP in time is relevant for the anti-arrhythmic studies and therefore extensively validated in chapter 2. Besides TdP incidence, the severity of arrhythmias was quantified as mean number of TdP per individual, TdP duration, mean number of defibrillations and number of ectopic activity.

For all presented anti-arrhythmic drugs, their effects were determined twice: as the ability to 1) suppress (table 2) and 2) to prevent dofetilide-induced TdP (table 3 and figure 2). As an important observation of these studies, it was noted that in both circumstances the antiarrhythmic effects of the drugs were quite similar (tables 2 and 3). In addition, the tables provide electrophysiological information how the anti-arrhythmic effects were achieved.

In the rank order of efficacy, flunarizine was the most efficient: it suppressed and prevented dofetilide-induced TdP completely. Similar efficacy was seen with verapamil, although in the prevention studies some TdP appeared after dofetildide (table 3). The anti-arrhythmic effects of ranolazine and lidocaine were relevant as TdP incidence was reduced by 60-67%. Finally with AVE0118 and K201 (lower dose), no significant anti-arrhythmic effects were seen (tables 2 and 3 and figure 2).

Table 2: Suppression experiments

		baseline	dofetilide	+ antiarrhythmic
Flunarizine	TdP (%)	0	100	0 #
	STV_{LV}	1.8±0.5	4.5±1.5 *	1.5±0.6 #
	QT_C	421±49	553±40 *	425±38 #
Verapamil	TdP	0	100	0 #
	STV_{LV}	1.7±0.4	3.2±1.1 *	1.5±0.7 #
	QT_C	424±62	566±87 *	516±90 *
Ranolazine	TdP (%)	0	100	40 #
	STV_{LV}	2.5±0.4	4.5±0.8 *	3.2±0.5 #
	QT_C	416±59	523±69 *	489±88
Lidocaine	TdP (%)	0	100	33 #
	STV_{LV}	1.4±0.3	3.7±1.2 *	2.3±0.4 #
	QT_C	360±51	478±41	494±75 *
K201, lower	TdP (%)	0	100	100
	STV_{LV}	2.0±0.5	3.8±1.0	Np
	QT_C	421±68	564±98	462±66 #
Ave0118	TdP (%)	0	100	100
	STV_{LV}	2.3±0.9	5.3±0.1 *	not possible
	QT_C	362±25	498±40 *	480±50*

*, p<0.05 vs baseline; #, p<0.05 vs dofetilide

Table 3: Prevention experiments

		baseline	antiarrhythmic	+dofetilide
Flunarizine	TdP (nr)	0±0	0±0	0±0
	STV_{LV}	1.5±0.6	1.0±0.5 *	1.4±0.5
	QT_C	413±51	369±41 *	476±77 #
Verapamil	TdP (nr)	0±0	0±0	0.2±0.4
	STV_{LV}	1.3±0.4	1.4±0.6	2.3±1.4
	QT_C	417±58	417±41	611±34 #
Ranolazine	TdP (nr/%)	0±0	0±0	3±3
		0%	0%\	33%
	STV_{LV}	1.8±0.9	2.0±0.7	3.3±1.8 #
	QT_C	395±94	438±104	565±76 #
Lidocaine	TdP (nr/%)	0±0	0±0	1.2±1.2
		0%	0%	33%
	STV_{LV}	1.3±0.6	1.2±0.6	3.1±1.0 #
	QT_C	381±81	318±49 *	586±93 #
K201, lower	TdP (%)	0	0	83 #
	STV_{LV}	1.1±0.5	1.3±0.7	4.0±0.7 #
	QT_C	400±50	470±54 *	666±94 #
Ave0118	TdP (%)	0	0	100 #
	STV_{LV}	2.1±0.4	2.1±0.3	4.6±1.8 #
	QT_C	438±47	438±44	500±74 #

*, p<0.05 vs baseline; #, p<0.05 vs antiarrhythmic drug

Electrical or surrogate ***bio-markers of anti-arrhythmic efficacy***

The reduced repolarization reserve of the dog with CAVB is characterized electrophysiologically by prolonged repolarization duration (acquired long-QT syndrome) and an increased baseline BVR[22] (chapter 3).
In the beginning of the thesis, we hypothesized that manipulating (decreasing or stabilizing) of BVR would result in antiarrhythmic effects (figure 3). Confirmation that this was indeed the case, as well in suppression as in prevention studies, is an important result of this thesis.

The pro-arrhythmic effects of dofetilide were preceded by an increase in STV_{LV} (chapters 2-7). Suppressive anti-arrhythmic interventions were previously shown to be linked to a

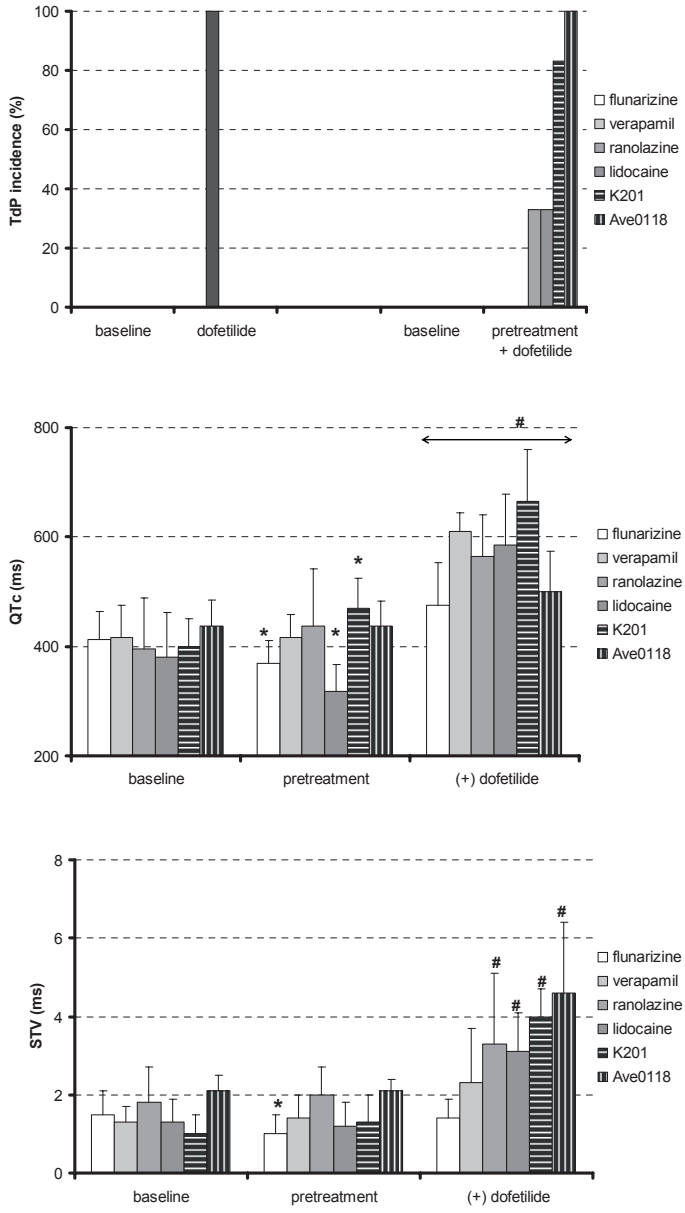

Figure 2 TdP prevention and surrogate parameters (QTc and STV_{LV})
Upper graph pictures the results of the prevention experiments. In these selected dogs, dofetilide induced TdP (100%), whereas pretreatment with different drugs had variable anti-arrhythmic effects (right part). The response of QTc (middle) and STV_{LV} (lower graph) in baseline, after pretreatment with the drugs and with addition of dofetilide is depicted. Please note that STV_{LV} reflects the (in)ability of the drugs to prevent TdP, whereas the behavior of QTc does not.

decrease in STV_{LV}[23]. Complete suppression of dofetilide-induced TdP with flunarizine and verapamil was associated by returning STV_{LV} to baseline levels, incomplete suppression with ranolazine and lidocaine with a significant reduction of STV_{LV} to intermediate values and a lack in anti-arrhythmic response with Ave0118 and K201 as no effect on STV_{LV} (figure 3, upper panel).

Similar results were seen with the prevention experiments (table 3): a (almost) complete prevention of TdP with flunarizine and verapamil was associated with a lack of increase in STV_{LV} and an incomplete prevention or no antiarrhythmic effect by a partial or full increase in STV_{LV} (figure 3, lower panel).

In contrast to STV_{LV}, QTc duration showed a much more variable behavior (tables 2 and 3). In spite of complete suppression of TdP after verapamil no significant shortening of the prolonged QTc was seen, whereas pretreatment with verapamil demonstrated anti-arrhythmic efficacy despite an increase in QTc duration (table 2 and figure 2). The other drugs of the suppression studies showed a full return to baseline (flunarizine), significant shortening of QTc (ranolazine and K201), and even no change in QTc-time (lidocaine). Such inconsistent behavior of QT in relation to arrhythmic outcome was also demonstrated for the prevention studies (table 3): dofetilide increased QTc duration in all cases with the largest increase seen after pretreatment with verapamil and K201 with almost complete or no prevention (figure 2, table 3).

The strongest anti-arrhythmic effects were seen with flunarizine. In addition, this drug was the only one who decreased baseline STV_{LV} (figure 2) and fully protected the heart from an increase in STV that is normally seen with dofetilide (table 3 and figure 3). The combination verapamil-lidocaine was able to decrease baseline STV in this model too, indicating that this combined block of I_{CaL} and I_{Na} is responsible for the effect of flunarizine (chapter 5).

In vitro, the anti-arrhythmic properties of flunarizine and ranolazine were also present against drug-induced EADs and linked to cellular APD and STV. Flunarizine reversed STV to baseline levels while with ranolazine the anti-arrhythmic effect was accompanied with a reduction to intermediate values.

Relevance and applications

1. In this thesis, it was shown that BVR, quantified as $STV_{LV,}$ may be used as a marker for repolarization reserve (chapter 2) and as such possibly used as a parameter to risk stratify patients. Additional studies are warranted to validate this concept, but the first positive signals have been shown in patients with heart failure. In this group, baseline STV_{QT} was increased compared to controls[24].
2. In addition, BVR changes follow both the pro-arrhythmic (an increase in STV_{LV}) and anti-arrhythmic properties of drugs. Especially the latter, reversing dofetilide-augmented STV_{LV} and prevention of STV_{LV} increase with pretreatment are new observations. Moreover, a decrease STV_{LV} in baseline after flunarizine was reported for the first time, which may be interpreted as an improvement in the repolarization reserve.
3. The dog with CAVB is a model of bradycardia induced volume overload and hypertrophy. The model has several similarities with other pre-clinical models or patients with prolonged repolarization times, lability of repolarization and an increased risk for ven-

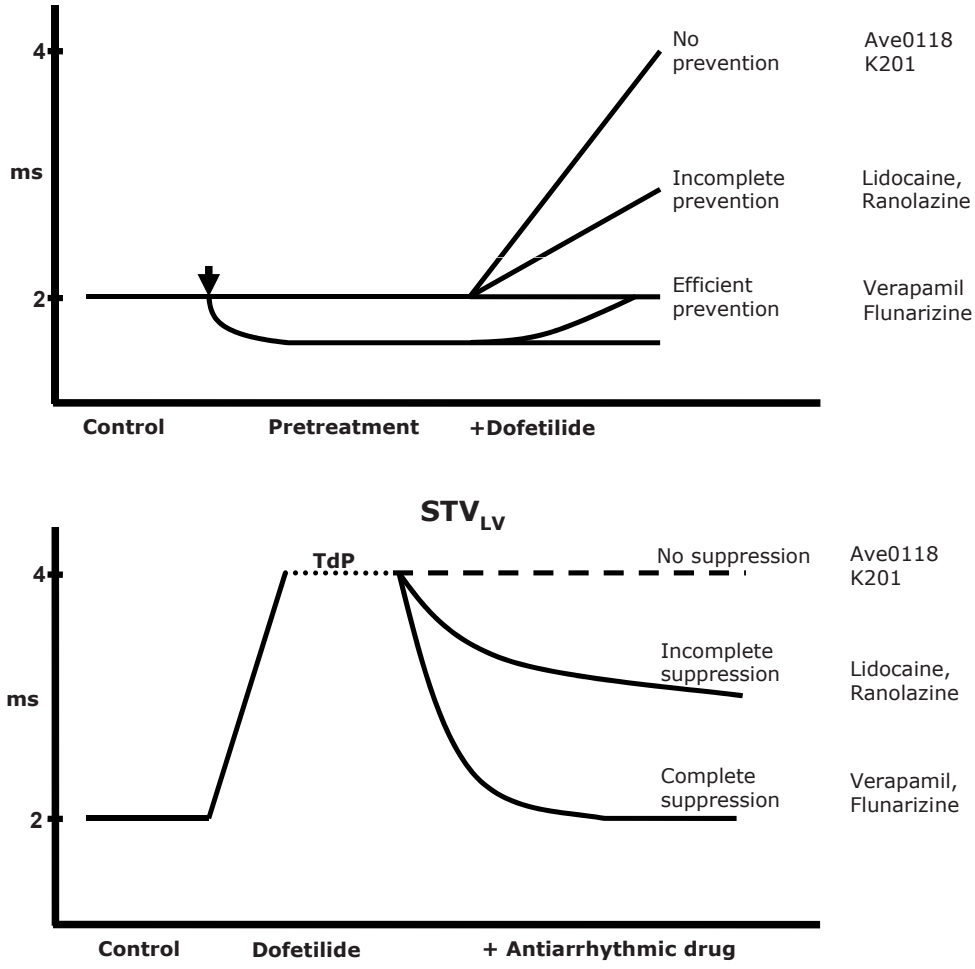

Figure 3: BVR manipulation and antiarrhythmic effects
STV_{LV} behavior (conceptual graphics) to explain the anti-arrhythmic interventions of the different drugs in this thesis: suppression (upper) and prevention (lower panel) against dofetilide-induced TdP. Hypothetical BVR behavior to explained the (in)ability of drugs to be antiarrhythmic.

tricular arrhythmias and sudden cardiac death[25-31]. Patients with congestive heart failure have a diminished repolarization reserve, an increased beat-to beat variability of repolarization duration (STV_{QT}) and a risk for sudden cardiac death due to downregulation of K^+ currents and calcium mishandling[24,32-34]. Other methods that quantify the lability of repolarization have also reported to be associated with an increased risk to sudden cardiac death[35-38].

4. In our CAVB model, L type Ca^{2+} antagonism without (verapamil) or in combination with late I_{Na} block (flunarizine) was the most efficient anti-arrhythmic strategy. Intermediate but still positive anti-arrhythmic effects were seen with late I_{Na} block (ranolazine, lidocaine) and no anti-arrhythmic effects were observed following K201, a presumed inhibitor of diastolic Ca-sparks (table 4).

Limitations

Negative inotropic effects of $I_{Ca,L}$ block are a contraindication in patients with heart failure and should therefore be avoided. All experiments were performed in total anesthesia and therefore the roll of adrenergic influences was limited. Not all the possible targets were explored, especially block of the NCX current is of interest.

Table 4: Conclusions

	Flunarizine $I_{Ca,L}$ + late I_{Na} ≥	Verapamil $I_{Ca,L}$ >	Ranolazine/Lido late I_{Na} >	K201 Ca-sparks
S=P	↓100 %TdP	↓ ≈100 %TdP	↓60-70% TdP	↓ 0-20% TdP
S	↓ STV to baseline	↓ STV to baseline	Partial ↓ STV	≈ STV
P	↓ baseline-STV	≈ baseline-STV	≈ baseline-STV	≈ baseline-STV
D	≈ STV	≈ STV	partial ↑ STV	↑ STV
S	↓ QTc to baseline	↓ QTc	↓/ = QTc partial	= /↓ QTc
P	↓ baseline QTc	= baseline QTc	=/ ↓ baseline QTc	=/ ↑ baseline QTc
+D	↑ QTc	↑↑ QTc	↑↑ QTc	↑↑ QTc
	↑↑↑ rep. strength	↑↑ rep. strength	↑rep. strength	= rep. strength

Conclusions

1. Pharmacologic control of BVR is antiarrhythmic against dofetilide induced TdP in the dog with CAVB. This is evident for repression of dofetilide induced increases in BVR (suppression), the observed reduction in baseline BVR and/or preventing an increase in BVR with dofetilide. Thus, BVR can quantify the anti-arrhythmic actions of drugs (figure 3).
2. BVR is a parameter that can quantify the repolarization reserve not only in pro-arrhythmic settings but also in baseline and during anti-arrhythmic conditions.
3. Combined block of $I_{Ca,L}$ and late I_{Na} by flunarizine is currently the most attractive pharmacologic approach against TdP (table 4), but this action is accompanied by a negative inotropic effect.
4. The results of the prevention or suppression studies with the different drugs were quite similar. So depending on the pharmacokinetics of the medication a choice how to determine anti-arrhythmic efficacy can be made without methodological concerns.

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English summary

The human heart is a biological pump that regulates the blood flow through the whole body. The rhythmic contraction of the heart muscle is due to electrical impulses from the pacemaker cells that spread to the rest of the myocardium. In every heart cell this electric impulse leads to changes in the membrane potential by activating ions currents that pass the cell membrane, phase labeled as depolarization which is followed by repolarization. These changes in voltage recorded in time form an action potential and all the action potentials of the heart cells form the basis of the electrocardiogram. This action potential produces an increase in the intracellular calcium concentration and so contraction takes place. Repolarization of the cell determines the restoring of the low intracellular calcium concentration which is the basis of relaxation needed for a new refilling of the heart with blood.

On the electrocardiogram measuring the QT interval (between Q wave and end T wave) one can gather information about repolarization. It is known that changes in the repolarization (e.g. increased repolarization duration like in genetic or acquired long QT syndromes) are the basis of dangerous ventricular rhythm disorders like Torsades de Pointes (TdP) and sudden cardiac death. Still the prolongation of the repolarization duration is not always predictive for such arrhythmias. As an alternative we have introduced beat-to-beat variability of repolarization (BVR) and shown that BVR is a better predictive parameter for drug-induced TdP than the QT interval.

In this thesis two hypothesis were investigated: 1) is BVR capable to detect high risk individuals in baseline (chapter 3) and 2) is BVR capable to determine possible antiarrhythmic effects of drugs, effects studied in relation to suppression in or prevention of drug-induced TdP (chapter 4-7).

Chapter 1 of this book is a general introduction, where the rational of this work is discussed.

Chapter 2 reviews the animal model used for the studies of this thesis, the dog with total AV block. By ablating the AV node in this model, the heart activates from a ventricular pacemaker and the heart rhythm becomes slow. To accomplish a normal circulatory function several cardiac adaptations occur that make the heart to become vulnerable to TdP and sudden cardiac death. Additionally we also studied in this chapter the repeatability and persistency of drug-induced arrhythmias showing that this animal model is suitable for pro- and anti-arrhythmic drug studies.

In ***chapter 3*** we studied BVR in several phenotypes: normal dogs, right after ablation of the AV node (acute AV block) and dogs with chronic AV block (at least 3 weeks later). Here we show that BVR is related to electrical remodeling. A normal heart has a stable repolarization duration, thus a low BVR. In chronic AV block baseline BVR is increased and the highest in individuals vulnerable for TdP. When these animals are challenged with a drug

(Dofetilide) that can induce TdP a further increase in BVR, in contrast to QT, is seen only when TdP occur. In such circumstances, in the following chapters we tried with several antiarrhythmic drugs to decrease and to stabilize the BVR in order to suppress and prevent TdP. These drugs were chosen for their action on specific ion currents in order to study their effect on BVR, their antiarrhythmic properties to suppress and to prevent TdP and their effects on other electrophysiological parameters (in particular QT).

In ***chapter 4*** the antiarrhythmic properties of a novel drug, AVE0118 are studied. As expected no antiarrhythmic effects and no effect on BVR were seen reason to use the results as a negative control.

Flunarizine and Verapamil are two known antiarrhythmic drugs and their efficacy against TdP was studied in ***chapter 5***. Both drugs block the calcium current. In our results both drugs were very efficient in suppressing and preventing TdP. These effects were reflected by BVR. We also studied other possible working mechanisms of these two drugs and demonstrated that they have a shared and different mode of action to explain their robust efficacy against TdP.

Chapter 6 presents the antiarrhythmic properties of Ranolazine and Lidocaine, both studied for their blocking effects on the natrium current. The natrium ion is involved in the function of the heart cell, both in depolarization and in restoring intracellular low calcium concentrations. In this study both drugs were able to partially suppress and to prevent TdP and again this property was the best seen in the effects on BVR.

The last drug studied in ***chapter 7*** is a new compound, K201. This drug was chosen for its intracellular effects against calcium release from the sarcoplasmic reticulum (SR). The SR is the cellular calcium deposit and it can be involved in the genesis of TdP. With this drug no antiarrhythmic effects was seen but surprisingly with the higher dose some pro-arrhythmic effects occurred reflected in an increase in BVR.

Finally, in the last part, ***chapter 8***, we integrated our results. The antiarrhythmic effects against TdP were best reflected by BVR, while changes in the QT parameter were variable. These studies bring new insights into the knowledge of ventricular polymorphic tachyarrhythmias, in particular TdP and which parameter can predict their risk. By reducing BVR in proarrhythmic circumstances we can suppress TdP and by reducing and stabilizing baseline BVR it is possible to prevent TdP. The robust antiarrhythmic effects were seen when the drugs could decrease completely proarrhythmic BVR back to baseline and could prevent an increase in BVR. Incomplete antiarrhythmic effects were reflected by an intermediate decrease or increase in BVR. In case of no antiarrhythmic property no effects on BVR were seen. Thus in this experimental setting by determining the BVR it is possible to study new drugs and to differentiate them in pro and anti-arrhythmic drugs by determining their effects on BVR.

Quantifying BVR implies a simple algorithm which could be applied in the clinic. By determining BVR it is possible to early detect patients at risk and by a proper treatment to prevent dangerous ventricular rhythm disorders and sudden cardiac death.

In conclusion with these studies we bring new insights in the diagnosis of such ventricular tachyarrhythmias (TdP) and strategies in their treatment and prevention.

Nederlandse samenvatting

Het hart is de spierpomp die zorgt dat het bloed door het hele lichaam stroomt. De ritmische pompfunctie van het hart wordt door elektrische prikkels bepaald. Deze prikkels beginnen in bepaalde delen van het hart (pacemaker cellen) en de elektrische impuls verspreidt zich dan naar de rest van het hart (twee atria en twee ventrikels). In elke hartspier leidt dit tot veranderingen in de membraan potentiaal: depolarisatie (geladen ionen passeren de celmembraan). Dit wordt gevolgd door repolarisatie (herstel van de ionen verplaatsing). Deze stroomveranderingen in tijd vormen een actiepotentiaal en alle actiepotentialen van de hartspiercellen zijn de basis van het elektrocardiogram (de hartfilm). Deze actiepotentiaal geeft in de cel een stijging van de calcium concentratie en dit veroorzaakt de samentrekking van de hartspier. Het herstel van de lage intracellulaire calcium concentratie in de cellen is verantwoordelijk voor de ontspanning en zo kan het hart weer met bloed gevuld worden.

Door het meten van het QT interval (lengte van Q-golf tot einde T-golf) op het elektrocardiogram kan men de informatie krijgen over de repolarisatie. Het is bekend dat veranderingen in de repolarisatie, verlengde repolarisatie (zoals in genetisch of verworven lange QT syndromen), de basis zijn van gevaarlijke ventriculaire ritmestoornissen waaronder Torsades de Pointes (TdP) en plotse hartdood. Echter, de verlenging van de repolarisatie duur alleen heeft niet altijd een voorspellende waarde voor zulke ritmestoornissen. Als alternatief hebben we recent aangetoond dat de variabiliteit van de repolarisatie duur (van slag-op-slag) een betere voorspellende parameter is voor TdP dan QT tijd.

Er zijn in dit proefschrift twee vragen onderzocht: 1) is slag-op-slag variabiliteit van de repolarisatie duur in staat om risico op TdP te voorspellen? (***hoofdstuk 3***) en 2) kan deze parameter de antiaritmische effecten van medicijnen voorspellen (***hoofdstuk 4-7***)?

Hoofdstuk 1 van deze scriptie is een algemene introductie. Hierin worden de doelen van dit proefschrift kort besproken.

Hoofdstuk 2 beschrijft uitgebreid het gebruikte experimentele diermodel, de hond met totaal AV blok en de complexe veranderingen die hierin optreden. In dit model worden de atria van de ventrikels elektrisch "gescheiden" (middels ablatie), waardoor het hartritme wordt overgenomen door een ventriculaire pacemaker die trager is. Dit leidt tot vele aanpassingen in het hart die ook de gevoeligheid voor TdP en plotse hartdood bepalen. Verder wordt in dit hoofdstuk ook het diermodel methodologisch onderzocht en bevestigd voor gebruik in onze antiaritmische studies (persistentie en herhaalbaarheid van de ritmestoornissen in tijd). Vervolgens is de waarde van de voorspellende parameters voor TdP beschreven.

In ***hoofdstuk 3*** hebben we de slag-op-slag variabiliteit van de repolarisatie bestudeerd in verschillende groepen honden: normale honden, honden kort na het induceren van een totaal AV blok en honden met een chronisch AV blok (AV blok van ten minste drie weken oud). Hier wordt aangetoond dat de variabiliteit van de repolarisatie gerelateerd is aan com-

plexe aanpassingen en veranderingen die optreden bij een chronisch AV blok. Een normaal hart heeft een zeer stabiele repolarisatie duur met een dus lage variabiliteit. De repolarisatie duur bij een hond met chronisch AV blok heeft een hoge slag-op-slag variabiliteit. Deze honden zijn getest met een medicijn dat TdP kan induceren (Dofetilide). In de groep honden die door dofetilide TdP kregen, werd gezien dat zij een hogere baseline variabiliteit van de repolarisatie duur hadden en dat deze variabiliteit verder steeg voordat TdP optrad. Deze stijging was alleen te zien in honden gevoelig voor TdP, in tegenstelling tot de QT tijd die in beide groepen was verlengd. In deze groep kwetsbaar voor TdP hebben we in onze studies geprobeerd met verschillende farmacologische middelen deze variabiliteit te verlagen en te stabiliseren om gevaarlijke ritmestoornissen te kunnen onderdrukken en te voorkomen. In de volgende hoofdstukken worden de elektrofysiologische effecten van een aantal farmacologische middelen bestudeerd. Deze middelen kunnen bepaalde ion stromen belemmeren, die betrokken zijn bij het functioneren van de hartspiercel, waardoor de variabiliteit van de repolarisatie kan worden beïnvloed.

In ***hoofdstuk 4*** worden de antiaritmische eigenschappen van een nieuw medicijn, AVE0118, getest. Bij dit middel werden geen antiaritmische effecten op het ventrikel gezien en ook geen effect op de variabiliteit van de repolarisatie, waardoor het verder als negatieve controle gebruikt kon worden.

Flunarizine en Verapamil zijn twee bekende antiaritmische middelen, die in ***hoofdstuk 5*** werden onderzocht op hun antiaritmische invloed op TdP. Beide middelen zijn bekend om hun remmende werking op de calcium stroom. In onze studies zijn deze middelen zeer effectief om TdP te onderdrukken en te voorkomen. Deze efficiëntie is gekoppeld aan effecten op de variabiliteit van de repolarisatie, herstel van de variabiliteit in suppressie en het voorkomen van een stijging in preventie experimenten. Verder hebben we deze twee middelen op andere cellulaire mechanismen van werking onderzocht waaruit blijkt dat beide additionele werking hebben die de antiaritmische efficiëntie kan versterken.

In ***hoofdstuk 6*** zijn de antiaritmische eigenschappen van Ranolazine en Lidocaine getest vanwege hun remmende werking op de natrium stroom. Natrium stromen zijn nauw betrokken bij de functie van de hartspier. Beide middelen onderdrukken of voorkomen partieel TdP en deze eigenschap was weer het best te zien in de effecten op de slag-op-slag variabiliteit van de repolarisatie duur.

Een andere complex werkend middel, K201, werd in ***hoofdstuk 7*** onderzocht. Dit middel werd gekozen om zijn intracellulaire werking op de calcium release vanuit het sarcoplasmatic reticulum (SR). Het SR is het calcium magazijn van de spiercel en kan betrokken zijn bij het ontstaan van TdP. Met dit medicijn hebben we helaas geen antiaritmische, maar zelfs enkele pro-aritmische effecten gezien, afhankelijk van de dosering, die gekoppeld waren aan een stijging van de variabiliteit.

In het laatste gedeelte (***hoofdstuk 8***) zijn alle uitslagen geïntegreerd besproken. De antiaritmische effecten van medicijnen tegen TdP waren het best te zien in hun effecten op de slag-op-slag variabiliteit van de repolarisatie duur, terwijl de veranderingen in QT parameter variabel waren.

Dit proefschrift levert een nieuw inzicht in deze ritmestoornissen, TdP, en welke parameter het beste het optreden hiervan kan voorspellen. Door het verminderen en/of stabiliseren

van de “baseline” slag-op-slag variabiliteit van repolarisatie, of door het onderdrukken van een verhoogde variabiliteit is het mogelijk aTdP te voorkomen of te onderdrukken. Ook het antiaritmische effect van verschillende medicijnen (compleet, incompleet en geen antiaritmische effect) was te zien aan veranderingen in de variabiliteit van de repolarisatie. Door het bepalen van deze nieuwe parameter is het mogelijk in deze experimentele setting nieuwe medicijnen te testen op pro- of anti-aritmische activiteit. Het kwantificeren van deze parameter (slag-op-slag variabiliteit van repolarisatieduur) heeft een eenvoudig algoritme, welke in de kliniek toegepast zou kunnen worden. Deze parameter kan gebruikt worden voor een risicostratificatie van een patiënten populatie. Hierdoor kunnen risicopatiënten vroegtijdig ontdekt worden zodat er met een behandeling voorkomen kan worden dat gevaarlijke ventriculaire ritmestoornissen en plotse dood ontstaan. Concluderend, met deze studies leveren we inzicht in nieuwe diagnostiek voor het voorspellen van het optreden van gevaarlijke ventriculaire ritmestoornissen, TdP, waardoor behandelingsmogelijkheden en/of preventiestrategieën vroegtijdig kunnen worden ingezet.

Rezumat in limba romana

Inima umana este o pompa biologica care recircula sangele in tot corpul. Contractia ritmica si sincrona a muschilor inimii este controlata de impulsuri electrice care pornesc din celulele "pacemaker" cardiace. In fiecare celula (cardiomiocit) acest impuls determina o schimbare de potential printr-un curent de ioni care traverseaza membrana. Aceasta faza care se numeste depolarizare este urmata de refacerea potentialului de repaus, faza numita repolarizare. Aceste schimbari de potential electric inregistrate in timp la suprafata corpului uman stau la baza inregistrarii electrocardiografice (ECG). Depolarizare induce un flux de ioni de calciu inspre interiorul celulei declansand astfel contractia. Repolarizarea celulei determina refacerea unei concentratii scazute de calciu in celula si astfel relaxarea are loc, necesara reumplerii inimii cu sange pentru un nou ciclu cardiac.

Adaptari ale procesului de repolarizare (de exemplu datorita unor boli sau a unor medicamente) stau la baza unor tulburari de ritm cardiac periculoase, de exemplu Torsade de Varfuri (international denumite Torsades de Pointes, TdP), care este o tahicardie ventriculara polimorfica. Cel mai des, informatii despre repolarizarea cardiaca se obtin dintr-o inregistrare ECG prin masurarea intervalului QT (intre unda Q si sfarsitul udei T, QT). Sunt bine cunoscute tulburarile de ritm care pot sa apara in cadrul sindromului QT lung (genetice sau dobandite), in care faza de repolarizare este prelungita (deficitara) situatie care poate degenera in aparitia torsadelor de varfuri, aritmie care poate evolua rapid in moarte subita. Totusi un interval QT prelungit nu este intotdeauna un parametru predictiv pentru astfel de tulburari de ritm. Ca si alternativa grupul nostru a introdus un nou parametru, variabilitatea repolarizarii (duratei de repolarizare) pe baza consecutiva (contractie dupa contractie) si a demonstrat ca acesta e un parametru cu o valoare predictiva mai buna decat QT pentru astfel de tulburarile de ritm.

In aceasta teza doua ipoteze au fost cercetate: 1) daca cuantificarea variabilitatii repolarizarii pe baza consecutiva este capabila sa detecteze persoanele la risc pentru TdP (capitolul 3) si 2) daca aceasta cuantificarea a variabilitatii repolarizarii este capabila sa determine posibilele efecte antiaritmice ale medicamentelor. Aceste efecte au fost studiate in relatie directa cu tulburarile de ritm, TdP (capitolul 4-7).

Capitolul 1 este o indruducere generala. Aici sunt explicate sumar scopurile acestei teze.

In ***Capitolul 2*** este descris modelul experimental: modelul canin cu bloc total atrio-ventricular. Prin ablatia nodului atrio-ventricular (AV), ritmul de contractie al inimii este preluat de un centru ventricular care are o frecventa mai mica. Ca sa-si mentina functia, inima trece printr-o serie de modificari complexe, adaptari care stau si la baza vulnerabilitatii pentru tachiaritmii ventriculare polimorfice (de ex. TdP) si a mortii subite. Aceste procese de remodelare sunt aici descrise amanuntit. Tot in acest capitol modelul animal este testat experimental si validat ca si potrivit pentru studiile antiaritmice ulterioare. In final in acest

capitol este discutata valoarea predictiva a parametrilor electrofiziologici studiati.

Capitolul 3 prezinta un studiu al variabilitatii repolarizarii in trei fenotipuri diferite de caini: un grup cu activitate cariaca normala (in rimtm sinusal), un grup de caini cu bloc acut AV totaal si un grup cu bloc AV total, cronic (dupa 3 saptamini de la ablatia nodului AV). In acest capitol este demonstrat ca varibilitatea repolarizarii se relateaza la modificarile cronice care apar in timp. In mod normal o inima sanatoasa are o variabilitate a repolarizarii foarte stabila deci foarte mica (<1 ms). In modelul nostru cu bloc total AV, cronic, variabilitatea repolarizarii este crescuta. Aceste grupuri de caini sunt testate cu un medicament care poate produce torsade de varfuri (TdP). Retrospectiv, dupa aparitia aceste tahyarimiei ventriculare (TdP), a devenit evident ca variabilitatea repolarizarii era cea mai mare in grupul de indivizi vulnerabili la TdP si in plus aceste tulburarile de ritm, sunt vazute doar dupa o crestere aditionala a variabilitatii. In contrast cu QT, variabilitatea repolarizarii creste doar in grupul sensibil la tulburarile de ritm (TdP). In astfel de circumstante, in studiile urmatoare, am incercat cu diversi compusi farmacologici sa controlam variabilitatea repolarizarii in speranta ca astfel vom putea suprima sau preveni aceste tulburarile de ritm ventriculare, TdP.

Asadar, in capitolele urmatoare sunt studiate efectele antiaritmice ale unor medicamente. Aceste substante chimice pot sa influenteze diversi curenti ionici celulari implicati in funcionarea normala a muchiului cardiac si astfel e posibil sa influenteze si variabilitatea repolarizarii. Efectele antiaritmice ale acestor compusi au fost studiate in prezenta TdP (supresie) sau innaintea aparitiei tulburarilor de ritm (preventie) si in relatie cu efectele lor asupra variabilitatii repolarizarii.

In ***capitolul 4*** sunt studiate proprietatile antiaritmice ale unui nou medicament cu nume de cod AVE118. Acest compus nu s-a dovedit antiaritmic la nivelul acestor tulburarilor de ritm ventriculare (TdP) si in acelasi timp nu s-a masurat vreo o modificare a variabilitatii repolarizarii, motiv pentru care in partea integrativa (capitolul 8) este folosit ca si control negativ.

Flunarizine si Verapamil sunt doua medicamente antiaritmice care sunt studiate in ***capitolul 5***. Amandoua au un efect de blockare a curentului de calciu. In studiile noastre aceste doua medicamente sunt foarte eficiente in a suprima si preveni TdP, pentru doza aleasa. Aceste efecte pozitive sunt cel mai bine reflectate de schimbarile in variabilitatea repolarizarii. In plus am cercetat pentru acesti doi compusi si alte mechanisme celuare aditionale de actiune.

Capitolul 6 prezinta efectele antiaritmice ale Ranolazinei si Lidocaine, aceste medicamente fiind studiate pentru efectul lor blocant al curentului de sodiu. Ionul de sodiu este implicat in functionarea celulei cardiace atat in depolarizare cat si in refacerea concentratiei intracelulare de calciu de la sfarsitul contractiei. Aceste doua medicamente sunt partial antiaritmice in acest model de studiu, atat in ce priveste supresia cat si preventia TdP iar aceste efecte sunt din nou vizibile la nivelul variabilitatii repolarizarii: aceasta este redusa incomplet sau preventia cresterii variabilitatii este de asemenea incompleta.

Ultimul medicament studiat in aceasta teza este prezentat in ***capitolul 7***. K201 care este un medicament nou, a fost ales in acest studiu pentru proprietatea lui de regla eliberarea de calciu din reticulum sarcoplasmic. Reticulum sarcoplasmic este depozitul intracelular de calciu necesar contractiei si in anumite circumstante e posibil ca eliberarea spontana de calciu

sa poata declansa aritmii ventriculare. Acest medicament in studiul nostru nu s-a dovedit antiaritmic iar aceste rezultate au fost reflectate de efectele asupra variabilitatii repolarizarii.

In final, in ***capitolul 8***, au fost integrate resultatele acestor studiilor. Efectele antiaritmice impotriva torsadelor de varfuri (TdP) au fost cel mai bine reflectate de schimbarile de variabilitate a repolarizarii in timp ce prelungirea parametrul QT a fost variabila. Aceste studii aduc perspective noi in intelegerea acestor aritmii ventriculare polimorfice (in particular torsadele de varfuri) si a parametrilor care pot sa prezica riscul de aparitie a acestor aritmii. Prin reducerea variabilitatii in circumstante proaritmice sau prin reducerea si stabilizarea variabilitatii in baseline putem suprima sau preveni torsadele de varfuri. Efectele antiaritmice robuste au fost consecvent asociate cu o reducere completa a variabilitatii sau o prevenire a cresterii variabilitatii, rezultatele antiaritmice incomplete au fost reflectate de o reducere moderata a variabilitatii sau o prevenire incompleta a cresterii variabilitatii iar lipsa proprietatilor antiaritmice sunt insotite de lipsa de efecte asupra variabilitatii repolarizarii. Deci in aceste circumstante experimentale prin determinarea variabilitatii repolarizarii in baseline este posibila studierea noilor medicamenter si diferentierea lor in pro- sau anti-aritmice.

Quantificarea variabilitatii repolarizarii implica un algoritm relativ simplu care poate fi extins si aplicat in ingrijirea medicala. Cu acest parametru se poate detecta pacientul vulnerabil si printr-un tratament adecvat se pot preveni aceste tachyaritmii ventriculare periculoase si moartea subita ca si consecinta a acestor aritmii.

In concluzie prin aceste studii grupul nostru de cercetare aduce noi cunostinte stiintifice importante diagnosticul acestor arimtii in ce priveste riscul aparitiei lor precum si strategii in tratamentul si preventia lor.

Acknowledgements

It is no cliché and I do not feel obliged to begin like this, it is just my simple conviction that my energy and motivation to do good research comes from my belief and thereby I thank God for His gifts and the balance he brought into my personal life. I thank Him that I could finish this professional "marathon".

My first contact with research was in my country, Romania, where I was doing a pediatric retrospective clinical study. In 2004 I moved to the Netherlands and I was eager to continue my medical route. The best what could happen to me was to ask advice to Marc. At that time he was next to many other functions, also the student advisor of my wife. He gave me the best advice I could receive, regarding a career. My affinity with internal medicine was the very first reason to enter the cardio-vascular research group of prof. dr. Marc Vos. Thus "beste" Marc, you meant these years enormously to me, regarding my professional formation. I have to say, your school was sometimes hard but during these years most of your critic was constructive and your advice turned to be most of the time fruitful. I have learned certainly not only to have a critical thinking but also to be systematic and to think more organized. Thanks for your patience and for your guidance! I really appreciate you for everything you have done for me. I appreciate your vocation for the positions you fill in and I can say it was a privilege to work in your team. If our work receives compliments than I have to say that you deserve most of them. I had a great time!

In the beginning of my work I was introduced into the "BVR" by Morten Thomsen. I appreciate you for you professional style and for your patience to teach me science. From you I have learned many skills and I was impressed how you worked with arguments. Thanks a lot for everything and I hope to see you soon as a professor.

Lot of time I have spent during these years with Jet Beekman, often in the operation room. Thanks for being so social! I felt your support and I am grateful for that! You have the vocation of a surgeon, keep going! I have learned lots of things from you! Thanks for everything!

Nico Attevelt, "beste" Nico we had a lot of fun in the operation room. Some projects we have done together and you know to cheer up people! Thank for you positive thinking and your support!

I want to thank to each one of my colleagues from these past years. During these four years working in a team was vital to our work. From this team my gratitude goes to each of you, individually, to Peter Oosterhoff , Stephan Winkels, Thom Stam, Roel van der Nagel, Gudrun Antoons, Marin Houtman, Mohamed Boulaksil, John Janssen and Marcel van der Heyden, thanks to each one of you for your input, I have learned a lot from our "brainstorms"! Your contribution to our research work was of great value. Also acknowledgments to Paul Volders and Theo van der Nagel for our collaboration in Maastricht! I want thank to everybody from the Medical Fysiology for all the scientific or personal discussions. Tony Woudenberg, dear Tony, thanks for listening!

I am sure there were more people with a direct or indirect contribution to this work, to all of you my sincere appreciation!

To my parents and parents in low, thank you for your interest and your questions and concern regarding my research work and its evolution, even if for you the most of the information was like a strange language.

And my last words are a public recognition to Imkje: thanks for being so supportive along these years! Thanks for working harder when I was too busy with research deadlines. Thanks for growing Simon so beautifully when I was more or less absent! This result was certainly possible with your “24 hours” support!

Curriculum Vitae

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List of common abbreviations

AV, atrio-ventricular;
AVB, atrio-ventricular block;
AAVB, acute complete atrio-ventricular block;
BVR, the concept of beat-to-beat variability of repolarization;
CAVB, chronic complete atrio-ventricular block;
CAVBr, CAVB resistant to drug-induced TdP;
CAVBs, CAVB susceptible to drug-induced TdP;
DAD, delayed after depolarization;
Δ MAPD, interventricular dispersion of repolarization, LV MAPD – RV MAPD;
EAD, early after depolarization;
ECG, electrocardiogram;
ERP, effective refractoriness period;
HW/BW, heart weight/body weight index;
LQTs, long QT syndrome;
LV, left ventricle;
MAP, monophasic action potential;
MAPD, monophasic action potential duration;
MEB, multiple ectopic beat;
PVT, polymorphic ventricular tachyarrhythmia;
RV, right ventricle;
SCD, sudden cardiac death;
SEB, single ectopic beat;
SR, sinus rhythm;
STV, short term variability, quantification of BVR;
TdP, Torsades de Pointes;
VF, ventricular fibrillation;
VT, ventricular tachycardia