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RESEARCH PAPER

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

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BACKGROUND AND PURPOSE

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

EXPERIMENTAL APPROACH

Our 11 year database of experiments of CAVB in dogs under general anaesthesia was reviewed and experiments included if ECG and LVP were recorded simultaneously at spontaneous rhythm. In total, 89 experiments in 44 dogs were appropriate and were analysed.

KEY RESULTS

During normally conducted sinus rhythm or acute atrioventricular block, EMW was positive. During CAVB, EMW was decreased to negative values. Dofetilide further reduced EMW before inducing repetitive TdP in 82% of the experiments. However, subclassification into inducible and non-inducible dogs revealed no difference in EMW. Analysis of the components of EMW revealed that the observed changes in EMW were solely caused by QT prolongation.

CONCLUSIONS AND IMPLICATIONS

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

Abbreviations

AAVB, acute complete atrioventricular block; AV, atrioventricular; CAVB, chronic complete AV block; DAD, delayed afterdepolarization; EAD, early afterdepolarization; EMW, electromechanical window; I_{Kr}, rapidly activating delayed rectifier potassium current; I_{Ks}, slowly activating delayed rectifier potassium current; LV, left ventricle; LVP, left ventricular pressure; Q-LVP_{end}, interval from QRS onset to the end of the LVP curve; QT_C, heart rate corrected QT interval; TdP, Torsade de Pointes

Introduction

Torsade de Pointes (TdP) is a life-threatening polymorphic ventricular tachycardia with typical twisting of the QRS complexes around the isoelectric line on the surface ECG. TdP often occurs in the setting of drug-induced QT prolongation and can be caused by both cardiovascular and non-cardiovascular drugs. The incidence of TdP with non-cardiovascular drugs is usually low (sometimes less than 1:10 000), which hampers early detection of this severe adverse event in clinical trials (Wysowski *et al.*, 2001; Pugsley *et al.*, 2008).

The heart rate corrected QT interval (QT_C) is the clinically most used surrogate parameter to assess risk of drug-induced TdP. QT_C is also important in safety pharmacology: analysis of the repolarization duration in animal models (ICH S7B) and a thorough QT study in humans (ICH E14) are important parts of the strategy to detect torsadogenic compounds before being introduced in the market (Pugsley *et al.*, 2008). A limitation is that the QT_C interval is not a good predictor of torsadogenic risk. Thus, drugs that prolong the QT_C can be free of TdP or even anti-arrhythmic and TdP can also occur in the setting of a short QT_C interval (Belardinelli *et al.*, 2003; Hondeghem, 2011).

Recently, Van der Linde *et al.* (2010) proposed the electromechanical window (EMW) as a new surrogate parameter for drug-induced TdP. EMW represents the interval between the end of the left ventricular pressure (LVP) curve and the end of ventricular repolarization (T-wave) and can be calculated by subtracting the QT interval from the interval from QRS onset to the end of the LVP curve (Q-LVP_{end}) (see Figure 1). Normally, the left ventricular (LV) contraction ends after repolarization, resulting in a positive EMW, which remains positive under dynamic physiological conditions (Boudoulas *et al.*, 1981; Van der Linde *et al.*, 2010). In anaesthetized dogs that were given isoprenaline in the presence of block of the slowly activating delayed rectifier potassium current (I_{Ks}) by HMR1556, Van der Linde *et al.* (2010) elegantly demonstrated that the duration of LVP and QT changed, in opposite directions. to create a negative EMW, which was related to the induction of TdP arrhythmias. In addition, they showed that TdP could be treated by drugs that made the EMW less negative.

This concept of EMW was suggested to be useful in guinea pigs, to detect pro-arrhythmic effects of other drugs, such as blockers of the rapidly activating delayed rectifier potassium current (I_{Kr}) and the L-type calcium current (Guns *et al.*, 2012a). Under general anaesthesia with pentobarbital, incremental dosages of the test compounds were administered. Drugs with a high risk of clinical TdP consistently caused a negative EMW, whereas drugs with no or low risk did not. For example, acute administration of amiodarone caused a greater increase of Q-LVP_{end} than of QT, thereby preventing negative EMW values.

In this study and also in another study conducted by Laursen *et al.* (2011), arrhythmogenesis in the form of early

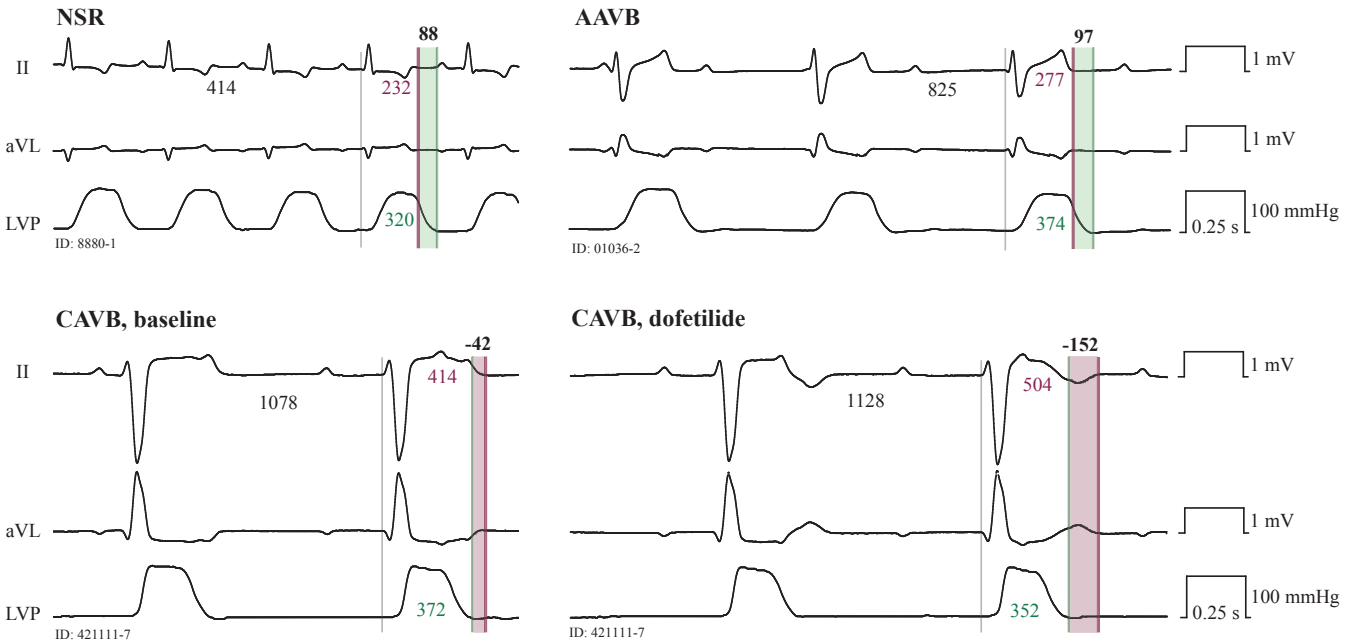

Figure 1

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

afterdepolarizations (EADs) or TdP was not a (reached) endpoint. However, in the latter study in Langendorff-perfused Göttingen minipig hearts, EMW values remained positive.

More recently, Guns *et al*. (2012b) reached the TdP endpoint in the unremodelled guinea pig using a combination of multiple pro-arrhythmic hits: anaesthesia, adrenaline pretreatment, the I_{Ks} blocker JNJ303, the compound of interest and, once more, adrenaline to trigger TdP. Using this approach, they were able to differentiate unsafe drugs from safe ones, at supratherapeutic concentrations. Although the EMW was more negative with the unsafe drugs, the precise contribution of Q-LVP_{end} and QT was not studied and the data suggested a close correlation of EMW with QT_C interval.

Ter Bekke *et al*. (2013) recently applied EMW in anaesthetized mongrel dogs with HMR1556-induced long QT1 in which the sympathetic nervous system was stimulated by either right- or left-stellate ganglion stimulation. Interestingly, only left-stellate ganglion stimulation induced TdP and this was linked to a more negative EMW than during right-stellate stimulation.

The chronic, complete atrioventricular block (CAVB) model is useful for testing pro-arrhythmic liability of drugs (see Oros *et al*., 2008). In groups that typically consist of 5–10 dogs, known torsadogenic drugs were successfully detected with induction of repetitive TdP arrhythmias as the primary endpoint. Drugs that are safe despite prolongation of repolarization, for example, amiodarone (Van Opstal *et al*., 2001b), were free of TdP in the model, indicating specificity in addition to the high sensitivity (see also overview in Stams *et al*., 2011). The increased susceptibility to drug-induced TdP of about 76% with the positive control drug dofetilide seems to be permanent and serial analysis of inducibility showed high repeatability during the first months after creation of atrioventricular (AV) block, when most experiments are performed in this model (Verduyn *et al*., 2001; Schoenmakers *et al*., 2003; Oros *et al*., 2008).

In this retrospective study, we addressed the following questions: (i) What is the effect of the electrical and contractile ventricular remodelling due to CAVB on the EMW? (ii) What is the effect of the I_{Kr}-blocking drug dofetilide on the EMW in the CAVB model? (iii) Is the EMW different in TdP-inducible and non-inducible dogs? (iv) Does the EMW have additional value over QT interval for TdP prediction in this model, based on analysis of the effect of these interventions on the electrical and mechanical components of the EMW?

Methods

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

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

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

A full description of the perioperative care, the procedure of AV-block creation and the signal processing is given by Van Opstal *et al*. (2001a) and Schoenmakers *et al*. (2003).

Data analysis

Electrophysiological parameters were analysed using the custom-made software that was used for the experiment (ECGView, Maastricht, the Netherlands; or ECG-Auto, EMKA Technologies, Paris, France; or EP Tracer, CardioTek, Maastricht, the Netherlands). At least five consecutive beats were used to calculate the mean for each parameter. The QT interval was measured from the onset of the QRS complex until the end of the T-wave in lead II using onscreen callipers and QT_C was calculated by Van de Water's formula (Van de Water *et al*., 1989). Q-LVP_{end} was measured similarly using the same beats, from the onset of the QRS complex until the end of the LVP curve (i.e. the end of the contractile force). EMW was calculated by subtracting QT from Q-LVP_{end}. The end-diastolic pressure, end-systolic pressure and maximum rise of the LVP per time unit (LV dP/dt_{max}), a measure of contractility, were also measured using the same software.

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

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

Statistical analysis

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

Results

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

A representative recording of ECG and LVP with calculation of EMW, as also described by Van der Linde *et al*. (2010), is shown in Figure 1.

Effects of AAVB and of remodelling due to CAVB

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

Effects of dofetilide in CAVB dogs

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

Subgroup analysis based on TdP inducibility

Stratification based on TdP inducibility revealed a significant difference in EMW at baseline, before administration of dofetilide in susceptible and non-susceptible animals (Table 3). Analysis of the components again revealed no shorter Q-LVP$_{end}$ in susceptible dogs, but only a longer QT interval (P < 0.01). Analysis of the other parameters also revealed a longer RR interval in the susceptible dogs and a higher LV end-diastolic pressure (P < 0.05). After administration of dofetilide, no significant differences were present any more for any of the parameters, including EMW (Table 3).

Amiodarone

In a previously published study (Van Opstal *et al*. (2001b) of CAVB dogs (6 weeks after AV-block creation; n = 7), chronic treatment with amiodarone (40 mg·kg^{-1}·day^{-1}) for 4 weeks resulted in a significant increase of QT from 340 ± 40 to 470 ± 75 ms (P < 0.05), but no induction of TdP. Q-LVP$_{end}$ was

Table 1

EMW determined at NSR, AAVB and CAVB

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

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

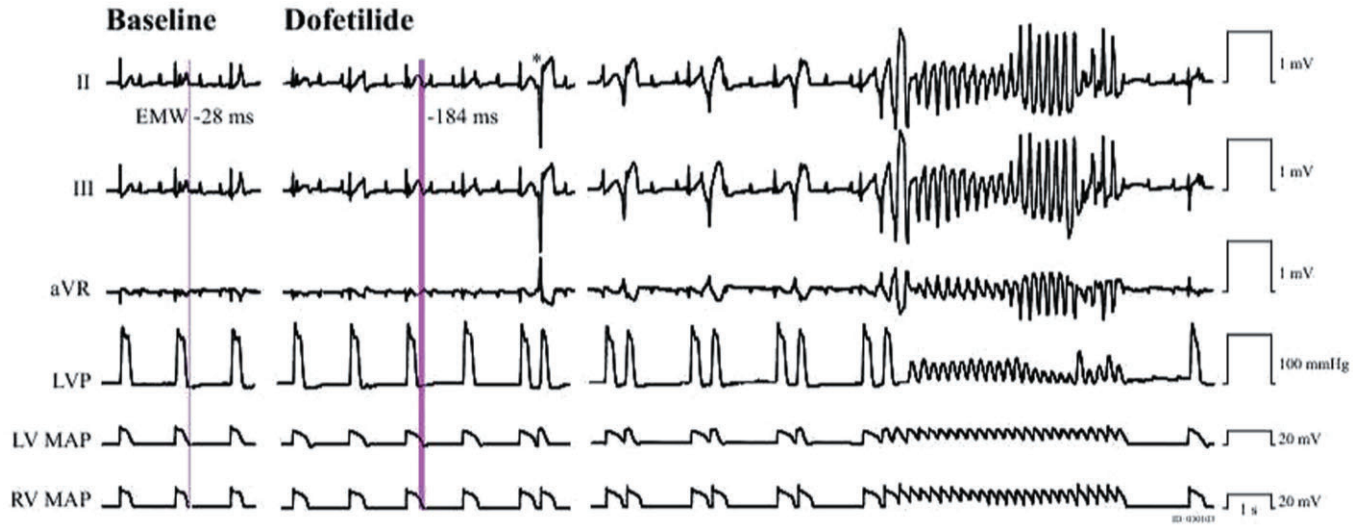

Figure 2

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

Table 2

Effects of dofetilide in CAVB dogs

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

Abbreviations are explained in the legend of Table 1. Only dogs where EMW was measured serially before and after dofetilide were included for this analysis (n = 38 experiments). ***P < 0.001, significantly different from Baseline; paired Student's t-test.

increased from 337 ± 21 to 366 ± 27 ms (P < 0.05), but this increase was not enough to prevent a reduction of EMW to negative values (from –5 ± 42 to –103 ± 54 ms, P < 0.01).

Components of EMW

Simple linear regression analysis after pooling all data from the individual experiments confirmed the close correlation of EMW with QT and the absence of correlation with Q-LVP$_{end}$ interval (Figure 3).

Detailed analysis

In 10 TdP-inducible CAVB dogs, we extended the EMW determination more closely (0.2 ± 0.2 min) before the occurrence of TdP. In these dogs, the original measurement before the first ectopic beat was performed at t = 3.0 ± 1.3 min, whereas the first TdP was seen, on average, 1.6 min later (range 0.2–5.2 min) at t = 4.5 ± 2.3 min. The extended analysis did not reveal differences in the parameters (all P > 0.1 using a paired Student's test): EMW (–151 ± 92 ms before TdP vs. –153 ± 36 ms before the first ectopic beat), QT (489 ± 88 ms vs. 484 ± 45 ms) and Q-LVP$_{end}$ (338 ± 42 ms vs. 332 ± 30 ms).

In only one of these dogs, we could detect low amplitude diastolic aftercontractions, which were not closely related to TdP. The absence of clear diastolic aftercontractions is also seen in Figure 2, where ectopic beats within the LV monophasic action potentials occurred.

Discussion

The main findings of this study were the following: (i) ventricular remodelling due to CAVB was linked to a decrease of EMW to values below zero; (ii) in CAVB dogs, dofetilide further decreased EMW to deep negative values, whether or not TdP was induced in the experiment; (iii) EMW reflected QT because Q-LVP$_{end}$ was not influenced by either the remodelling or by dofetilide; (iv) although chronic amiodarone treatment resulted in a small increase of Q-LVP$_{end}$, EMW reached clearly negative values, despite the absence of TdP induction.

Conditions affecting TdP (may) differ between the models

For use in safety pharmacology, EMW was initially described in β-adrenoceptor-provoked, tachycardia-dependent, long QT1 circumstances, mimicked by the blockade of I_{Ks} through HMR1556 in normal (unremodelled) anaesthetized

Table 3

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

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

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

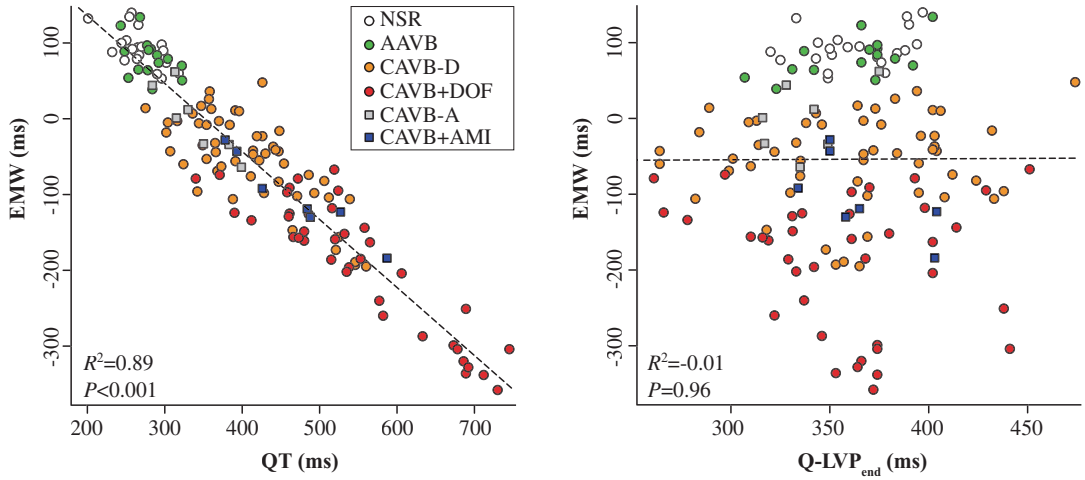

Figure 3

The contribution of the components of EMW, QT and the interval from QRS onset to end of the LVP curve (Q-LVP$_{end}$), using the pooled data of all experiments (normally conducted sinus rhythm, acute and chronic AV-block, dofetilide and amiodarone). Simple linear regression analysis (dashed lines) revealed that EMW correlates well with QT (left), with an adjusted R^2 value of 0.89 and a slope of –0.90 (95% confidence interval of the slope: –0.96 to –0.85), but not with Q-LVP$_{end}$ (right). This is in agreement with the comparisons of the groups (Tables 1 and 2). Data are shown from experiments with normally conducted sinus rhythm (NSR), acute AV block (AAVB), chronic AV block at baseline before dofetitlide (CAVB-D) and chronic AV block after dofetilide (CAVB+DOF); also experiments with chronic AV-block before amiodarone (CVAB-A) and chronic AV block after chronic amiodarone administration (CAVB+AMI).

(fentanyl-etomidate) dogs (Gallacher *et al.*, 2007; Van der Linde *et al.*, 2010). Initiation of TdP was preceded by a shift towards severely negative EMW values that returned to almost control values after anti-arrhythmic therapy with verapamil or atenolol. EMW changed (becoming negative) because the QT interval initially was increased after HMR1556 and, subsequently, Q-LVP$_{end}$ was decreased with the addition of isoprenaline, without further increase of QT, although QT$_C$ (not relevant for EMW calculation) did increase. Thus, it was crucial that the QT duration was unable to shorten upon the increase in heart rate after isoprenaline, because I_{Ks} was blocked.

The dog CAVB model was not developed to mimic a clinical, monogenetic long QT syndrome, but was a model of acquired long QT interval. In this model, compensated hypertrophy and numerous adaptations in ion channels and calcium handling proteins have been reported (Oros *et al.*, 2008),including down-regulation of the delayed rectifier potassium currents, increase of the sodium-calcium exchange current and increased calcium content of the sarcoplasmic

reticulum, which provides larger calcium transients, and an increase of LV contractility parameters (Volders *et al.*, 1999; De Groot *et al.*, 2000; Sipido *et al.*, 2000). Besides the ventricular remodelling, the anaesthetic regimen is a key ingredient for TdP induction (Dunnink *et al.*, 2010).

Stimulation of β-adrenoceptors and increasing heart rate, as applied in the other studies of EMW, were not part of the standard methodology to induce TdP in the CAVB model, but sudden rate accelerations by programmed electrical stimulation have been applied to increase the inducibility of drug-induced TdP (Vos *et al.*, 1995; Stams *et al.*, 2011). On the contrary, single injections of adrenaline did not increase inducibility and continuous pacing at a higher basal heart rate and isoprenaline administration have been applied to reduce the incidence of TdP (Vos *et al.*, 1995; Van Opstal *et al.*, 2001b; Oosterhoff *et al.*, 2010).

Mechanisms of TdP

Extensive efforts have been made to elucidate the underlying mechanisms of TdP, but the arrhythmogenesis of TdP in general and in the CAVB dog, are still not fully understood. EADs and delayed afterdepolarizations (DADs) are considered the primary cause of focal activity responsible for the initiation of the arrhythmias, although re-entry may also be involved especially during the perpetuation (Belardinelli *et al.*, 2003; Boulaksil *et al.*, 2011).

In the CAVB dog, anti-arrhythmic effects against TdP have been demonstrated by inhibition of EADs or DADs, for example, using the calcium channel antagonists verapamil and flunarizine (De Groot *et al.*, 2000; Oros *et al.*, 2010). In a recent study, the anti-arrhythmic agents verapamil and SEA-0400 (n = 3 for each drug) successfully suppressed dofetilide-induced TdP in this model (Bourgonje *et al.*, 2013). This effect was neither accompanied by shortening of the QT/QT_C interval (Bourgonje *et al.*, 2013) nor by changes of Q-LVP_{end}, thereby resulting in maintenance of a strongly negative EMW (–318 ± 17 ms after dofetilide + verapamil and –342 ± 49 ms after dofetilide + SEA-0400).

The initiation of DADs has been related to spontaneous calcium release from the sarcoplasmic reticulum during diastole. The distinction between DAD and EAD is only based on appearance in time within the repolarization (action potential) or not, and is not based on the underlying mechanisms. Therefore, they may share the same mechanism. In a long QT2 rabbit model, oscillations in intracellular Ca^{2+}, most likely caused by spontaneous Ca^{2+} release from the sarcoplasmic reticulum, preceded the oscillations in membrane potential and EADs and seemed to be driven by the increase of intracellular Ca^{2+} (Nemec *et al.*, 2010). However, in the setting of a prolonged repolarization duration, window currents, especially of the L-type calcium current, provide an additional mechanistic explanation for EADs not only in phase 2 but also in phase 3 of the action potential (Weiss *et al.*, 2010; Zhao *et al.*, 2012). The window currents are enhanced in left ventricular cardiomyocytes from CAVB dogs and are increased further by β-adrenoceptor stimulation (Antoons *et al.*, 2007). Only recently, distinctions between sarcolemmal and sarcoplasmic reticulum-dependent EADs have been documented by the difference in rate and their response to drugs and dynamic action potential clamp (Zhao *et al.*, 2012). However, these researchers also postulated that these mechanisms may act synergistically.

Aftercontractions and spontaneous Ca^{2+} release

In the intact heart, the LVP can be used to time-dependently relate mechanical information to electrical effects and ectopic activity. Intriguingly, Gallacher *et al.* (2007) reported that mechanical diastolic aftercontractions in the LVP seemed to precede EADs in the monophasic action potential recordings prior to TdP occurrence, suggesting that these aftercontractions may be caused by spontaneous Ca^{2+} release from the sarcoplasmic reticulum. Due to the negative EMW, these diastolic aftercontractions do not induce DADs but EADs in the action potentials and when triggering ectopic beats within the QT interval, the R-on-T phenomenon.

A limitation is that DADs or EADs can be relatively easily generated in cells, whereas they are much more complicated in the intact animal. A monophasic action potential is only recorded locally and restricted in its visualization because of electrical coupling of the myocytes. Due to the source-sink relations, spatiotemporal synchronization of EADs or DADs is required before afterdepolarizations can be visualized and focal activity can be induced and additional factors including β-adrenoceptor stimulation can influence this process (Myles *et al.*, 2012). The aftercontractions also raise the interesting possibility of mechanoelectrical feedback via stretch-sensitive ion currents as a cause of the EADs and arrhythmogenesis of TdP (Ter Bekke and Volders, 2012).

Implications for the application of EMW in safety pharmacology

As described earlier, we found no clear additional value of EMW over QT in the CAVB model as surrogate for drug-induced TdP and the measured QT prolongation after drug administration was not strongly associated with TdP inducibility (Van Opstal *et al.*, 2001b; Belardinelli *et al.*, 2003; Thomsen *et al.*, 2006; Stams *et al.*, 2011). As a consequence, other parameters may be more useful, obviously not only the arrhythmogenic outcome itself but also other surrogate parameters. Particular attention should be paid to the beat-to-beat variability of repolarization, quantified as short-term variability of the LV monophasic action potential duration, because this parameter has been reported to be better than QT and QT_C in the CAVB model, (see Oros *et al.* (2008) and Varkevisser *et al.* (2012). Also, the combination of cardiac wavelength, triangulation, reverse use dependence, instability and dispersion (the λ-TRIaD) provides an advantage over QT_C, as described by Hondeghem (2011).

The apparent lack of specificity of the EMW does not detract from its ability to depict the substrate, as TdP was consistently preceded by negative EMW values. This is in accordance with the other models and Ter Bekke and Volders (2012) have already discussed that EMW most likely is not the *primum movens*. However, our results imply that a negative EMW is not always pro-arrhythmic, and for safety pharmacology purposes, other parameters related to the actual triggering of arrhythmias episodes need to be assessed as well.

Study limitations

The relevance of the EMW was studied in this particular dog model of dofetilide-induced TdP under our standard conditions, which may limit extrapolation to other models and humans. These include (i) the anaesthetics used prolong repolarization duration and negatively affect the LVP; (ii) the origin of idioventricular rhythm is not controlled, leading to different activation patterns; (iii) the degree of bradycardia after AV-block is not controlled; (iv) reproducibility in the serial experiments is not complete, precluding 100% segregation in inducible and non-inducible animals. Only two drugs were studied for pro-arrhythmia: dofetilide (our gold standard to induce TdP) and amiodarone, which causes QT prolongation without induction of TdP.

We did not assess the effects of dofetilide on the EMW at AAVB because LVP recording during dofetilide was only available in one experiment, in which EMW remained positive and TdP was not induced, which is in agreement with earlier experiments (Thomsen *et al.*, 2007). The present study has a retrospective design, which may be more susceptible to bias, although only data from prospectively performed studies were used, with similar offline analysis of the data. Due to the limited amplitude resolution of the recording system on-screen and the presence of AV-block (interference by P-waves), reliable observation of aftercontractions was only possible if the amplitude exceeded about 10 mmHg.

In conclusion, in the canine CAVB model, ventricular remodelling and I_{Kr} block by dofetilide were associated with negative EMW values, but this closely reflected QT prolongation. We found no difference in the EMW after dofetilide between experiments with and without TdP arrhythmias. Chronic amiodarone treatment also decreased the EMW to negative values, although TdP arrhythmias were absent. Therefore, we conclude that in this CAVB model, TdP arrhythmogenesis was linked to negative EMW values, but the effects of dofetilide and amiodarone implied that the EMW had the same limitations as the QT prolongation as it lacked specificity to predict drug-induced TdP in the CAVB model in dogs.

Conflicts of interest

None.

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