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Differential effects of human ether-a-go-go-related gene (HERG) blocking agents on QT duration variability in conscious dogs

Johannes Schneider*, Renate Hauser, Jens-Otto Andreas, Klaus Linz, Ulrich Jahnel

Grünenthal GmbH, Department of Safety Pharmacology, Zieglerstrasse 6, 52078 Aachen, Germany

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Abstract

The effects of drugs that inhibit human ether-a-go-go-related gene (HERG) related cardiac potassium channels on the variability of QT duration as a sign of repolarisation instability were evaluated in conscious telemetered dogs. QT duration variability was determined using a beat-to-beat analysis before and after the infusions of HERG channel blocking agents. Variability was evaluated as increased mean width (P_{width}) and length (P_{length}) of Poincaré plots of 100 consecutive beats. As HERG channel blockers which are associated with arrhythmias of the torsades de pointes (TdP) type, dofetilide and sotalol were infused. Verapamil was used as an HERG channel blocker that is not associated with TdP. Dofetilide (0.01 and 0.03 mg/kg) dose-dependently prolonged QT_c duration (12% and 16%). Dofetilide also induced an increase of QT variability that reached statistical significance for P_{length} at the higher dose (64%). A dose of 3 mg/kg sotalol neither prolonged QT_c duration nor QT duration variability. In contrast, at 10 mg/kg sotalol prolonged QT_c duration (15%) and increased P_{length} (33%). Doses of 0.1 and 0.3 mg/kg verapamil did not increase QT_c duration nor QT time variability. QT duration variability in conscious dogs may be a useful preclinical marker to discriminate pro-arrhythmogenic and non-arrhythmogenic activities of HERG blocking agents.

Keywords: QT interval; HERG; Risk assessment; (Dog)

1. Introduction

A variety of drugs including anti-arrhythmics, antihistamines, antipsychotics and miscellaneous agents can induce QT interval prolongation. Most of these agents prolong cardiac repolarisation by inhibiting the rapidly activating delayed rectifier potassium current (I_{Kr}) encoded by the *human ether a-go-go related-gene* (HERG). Drug-induced QT interval prolongation is frequently associated with potentially fatal arrhythmias of the torsades de pointes (TdP) type (Haverkamp et al., 2000), leading to the withdrawal of some QT-prolonging drugs from the market. Any potential pro-arrhythmic activity is also of great concern in the pharmaceutical development of new therapeutic agents. Regulations thus require the evaluation of effects of new chemical entities on HERG activity and on QT interval prolongation (Shah, 2002).

However, the predictive value of preclinical QT interval prolongation is doubtful. Prolongation of the QT interval or the action potential duration itself is anti-arrhythmic and is the underlying mechanism of class III anti-arrhythmic agents (Brendorp et al., 2002). Thus, other factors must coincide to enable QT interval prolongation to become a pro-arrhythmic event. Spatial or temporal heterogeneity of cardiac repolarisation may increase the pro-arrhythmic risk. Studies on rabbit hearts suggest that an increase in transmural or interventricular dispersion of repolarisation contributes to the pro-arrhythmic and torsadogenic risk of QT interval prolonging agents (Eckardt et al., 2002). Temporal instability of the repolarisation phase of the action potential, which describes the heterogeneity of action potential durations from one cycle to the next, was found to be an important determinant of pro-arrhythmia in female rabbit hearts.

* Corresponding author. Tel.: +49 569 2423; fax: +49 569 2852.
E-mail address: Johannes.Schneider@grunenthal.de (J. Schneider).

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Among agents with class III anti-arrhythmic or HERG channel blocking activities, those also producing repolarisation instability had a higher propensity to be pro-arrhythmic. Thus, the induction of in vitro instability of action potential duration seems to be a good predictor to discriminate agents documented to have a pro-arrhythmic potential in clinical practice from those that are believed not to be pro-arrhythmic (Hondeghem and Hoffmann, 2003; Hondeghem et al., 2003). In view of the predictive value of in vitro instability of action potential duration, the evaluation of in vivo instability of the QT interval might add useful information to the pro-arrhythmic risk assessment of QT interval prolonging agents. In dogs fitted with telemetric devices, the variability of QT time was thus measured in a beat-to-beat electrocardiographic (ECG) analysis. QT duration variability was determined before and after the administration of HERG blocking agents believed to be pro-arrhythmic such as dofetilide (Weerapura et al., 2002; Van Opstal et al., 2001) and sotalol (Numaguchi et al., 2000; Weissenburger et al., 1991). Verapamil was studied as a HERG blocker with no or very low pro-arrhythmic potential (Zhang et al., 1999; Fossa et al., 2002; Redfern et al., 2003). The results suggest that the QT time variability may be a useful additional preclinical in vivo surrogate parameter to assess the risk of HERG blocking agents.

2. Materials and methods

2.1. Animals

Six male Beagle dogs (Harlan France SARL, Gannat, France) were used for the study. The animals were housed in individual boxes, but were allowed to move freely in groups of 1–2 h/day. The dogs were fed a standard laboratory diet (200–400 g/day) in the form of pellets. Tap water was freely available via an automatic system. A health check was performed after delivery and before being used in the experiment. Each dog was treated repeatedly with vehicle or different doses of the test substances at intervals of at least 7 days.

2.2. Telemetric measurements of blood pressure, heart rate and ECG

2.2.1. Telemetric devices

Sensors of the type TL11M2-D70-PCT to record blood pressure and ECG signals were implanted. Signals were picked up by RMC-1 receivers and transferred via the Dataquest ART exchange matrix and Dataquest Open ART Acquisition Interface to the Ponemah digital acquisition analysis and archive system software. Sensors, receivers and exchange matrix were obtained from Data Sciences International, St. Paul, Minnesota, the acquisition and analysis software from Gould, Valley View, Ohio.

2.2.2. Surgical implantation of transmitters

General anaesthesia was induced by intramuscular injection of 2 mg/kg xylazine HCl (Rompun 2%, Bayer, Leverkusen, Germany) and 10 mg/kg ketamine HCl (Kemint, Alvetra, Neumünster, Germany). Xylazine injections were repeated when required.

The left flank, the left and right thorax, and the sternal region were shaved. The left abdominal cavity was opened and a pressure and biopotential sensor implanted. A fluid-filled catheter of the sensor was inserted into the iliac artery and passed into the abdominal aorta for blood pressure measurement. The two biopotential leads were attached subcutaneously to the left upper thoracic wall and to the tip of the sternum to record a lead II ECG. After surgery, the dogs were treated with an antibiotic (Tardomyocel®) on alternate days for 1 week.

2.2.3. Data recording and acquisition

For the experiment, the unrestrained conscious dogs were allowed to move freely in a 1×1×1 m stainless-steel cage (Ebeco, Castrop Rauxel, Germany). Two receivers for the sensor signals were placed on both sides of the cage. The ECG and blood pressure signals were continuously monitored at a sampling frequency of 2000 Hz using the Ponemah system. The recorded and acquired pressure and ECG signals were analysed automatically using the Ponemah software. The analysis of the ECG

Table 1
Baseline values of mean arterial blood pressure, heart rate, QT and QT_c durations in conscious dogs (n=6; data as mean±S.E.M.)

Treatment	Dose [mg/kg]	Mean arterial blood pressure [mm Hg]	Heart rate [beats/min]	QT [s]	QT_c [s]
Solvent 1	–	117±3	91±11	0.211±0.007	0.231±0.004
Solvent 2	–	118±4	89±2	0.214±0.003	0.236±0.005
Solvent 3	–	117±3	86±7	0.217±0.007	0.235±0.003
Dofetilide	0.01	119±4	88±7	0.217±0.005	0.237±0.006
	0.03	124±6	83±4	0.218±0.007	0.236±0.005
Sotalol	3.0	117±5	84±4	0.221±0.006	0.239±0.004
	10.0	120±5	88±9	0.214±0.018	0.239±0.011
Verapamil	0.1	127±6	94±8	0.211±0.007	0.237±0.004
	0.3	123±5	97±9	0.207±0.006	0.233±0.003

Solvent 1: saline; solvent 2: 1% DMSO+5% glucose; solvent 3: saline titrated to pH of 4.7.

segments was checked manually in random samples in the offline mode of the system on completion of an experiment. The data were entered into a special custom-built application based on the Excel 97 programme for tabular calculations and presentations.

2.2.4. Experimental protocol

A 30-min equilibration time was allowed for adaptation to the cage. Three pre-values were then recorded for the following parameters: systolic, diastolic and mean arterial blood pressure, heart rate, RR interval, PR, QRS and QT times. The QT time was corrected for changes in heart rate using the formula $QT_c=QT-0.087(RR-1000)$ as described by Van de Water et al. (1989). The mean values of these pre-values taken at 15, 10 and 5 min before start of i.v. treatment were calculated as baseline values. These parameters were evaluated repeatedly at different times up to 120 min after the start of treatment. For each evaluation time, 10 complete blood pressure and ECG signals were recorded and averaged.

A repeated measurement analysis of variance with the factors animal and drug dose was performed to analyse the drug dose effect on heart rate and QT_c. If an overall drug dose effect over time was detected an analysis of

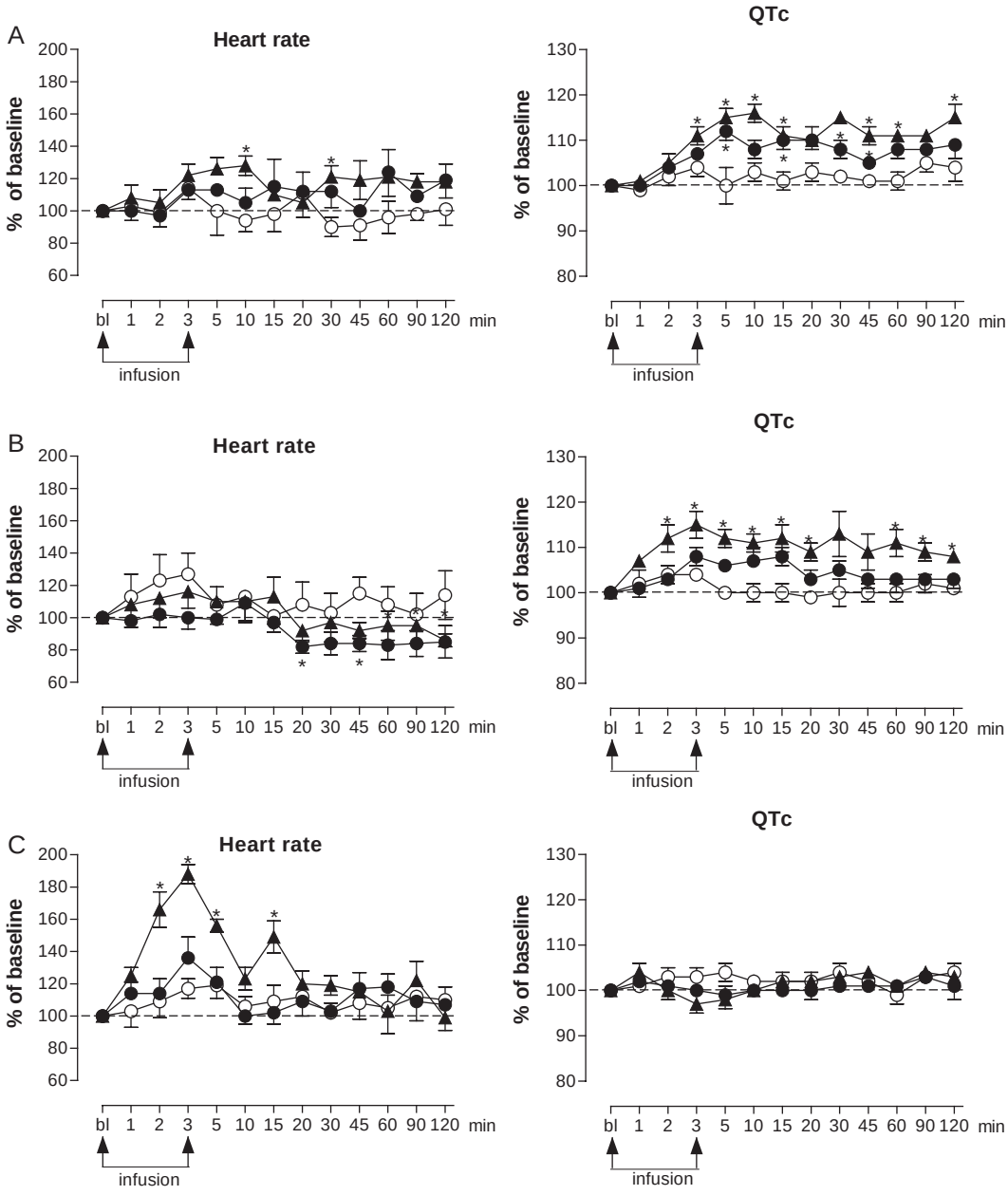

Fig. 1. Effects of intravenous infusions of dofetilide (A), sotalol (B) or verapamil (C). Low (●) and high (▲) doses are 0.01 and 0.03 mg/kg for dofetilide, 3.0 and 10.0 mg/kg for sotalol and 0.1 and 0.3 mg/kg for verapamil. (○) indicates vehicle in control groups. Data are given as means±S.E.M. of n=6 experiments per group. * Denotes statistically significant difference compared to time-matched vehicle values, $p<0.05$.

variance with the factors drug dose and animal was performed for each time point. The level of significance was set at $p<0.05$.

2.2.5. Beat-to-beat analysis

In addition to the evaluation of the parameters described in the experimental protocol, a beat-to-beat analysis of the ECG was performed 10 min before and 10 min after the start of the treatment infusions. At these times, QT values of 100 consecutive cycles were recorded and listed in an Excel sheet. Poincaré plots with QT_n versus QT_{n+1} values [ms] (i.e. QT value of a given cycle n versus QT value of the subsequent cycle $n+1$) were prepared for each of the two beat-to-beat analysis times. The mean width (P_{width}) of the plots, i.e. the mean orthogonal distance from the diagonal to the points of the Poincaré plot, was determined as $P_{\text{width}} = \sum |QT_{n+1} - QT_n| / [100 \times \sqrt{2}]$. The mean length ($P_{\text{length}}$) parallel to the diagonal of the Poincaré plot was calculated as $P_{\text{length}} = \sum |QT_{n+1} + QT_n - 2QT_{\text{mean}}| / [100 \times \sqrt{2}]$. P_{width} and P_{length} are regarded as parameters to indicate the variability of the Poincaré QT plots. These measures of Poincaré plot geometry have been described for heart rate variability investigations in humans (Brennan et al., 2001).

The P_{width} and P_{length} values of the QT Poincaré plots of each treatment group at baseline (before treatment) were compared to those obtained after treatment by means of the paired t-test. The level of significance was set at $p<0.05$. For statistical analyses, the Analyse-It software (Analyse-It Software, Leeds, UK) was used.

2.2.6. Pharmacological agents

Dofetilide (Key Organics, Camelford, UK) was dissolved in 1% dimethylsulphoxide (DMSO) and 5% glucose solution. D,L-Sotalol infusion solution was prepared from Sotalex® i.v., 40 mg ampoules (Bristol Myers Squibb, Munich, Germany). Verapamil (Research Biochemicals Int., Natuck, MA) was dissolved in physiological saline. All agents were infused intravenously over 3 min. The controls received 1% DMSO and 5% glucose solution, physiological saline titrated to pH of 4.7 with acetic acid or saline alone. These solvents did not affect blood pressure or the ECG parameters including the QT_c length.

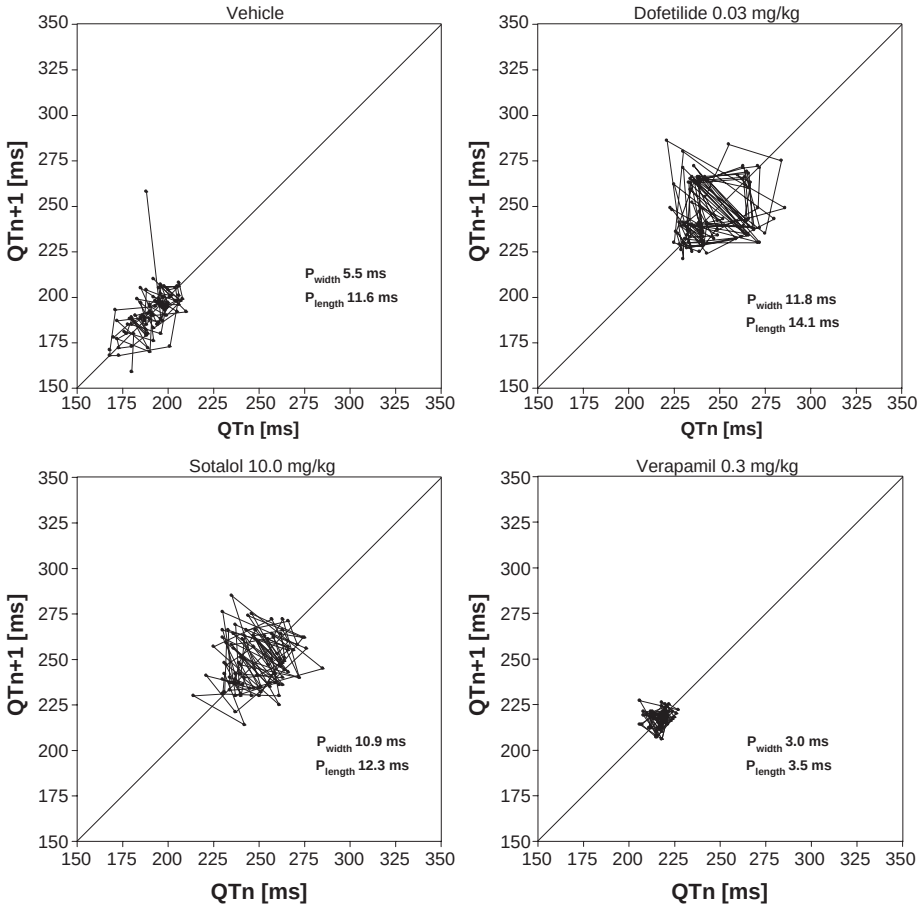

Fig. 2. Examples of beat-to-beat Poincaré plots of QT duration in one cycle (QT_n) to its subsequent cycle (QT_{n+1}) in the same individual dog. Hundred cycles each were evaluated at 10 min after start of infusions with vehicle, dofetilide 0.03 mg/kg, sotalol 10.0 mg/kg or verapamil 0.3 mg/kg.

3. Results

3.1. Effects of vehicle, dofetilide, sotalol and verapamil on heart rate and QT_c duration

Heart rate, QT and QT_c durations did not differ significantly between the treatment groups at baseline (Table 1). Infusion of 0.01 and 0.03 mg/kg dofetilide had no effect on heart rate. There was a dose-dependent increase in QT_c duration after both doses of dofetilide. After infusion of 3.0 and 10.0 mg/kg sotalol heart rate decreased after the low dose only, whereas after the high dose there was an increase in QT_c duration. Infusion of 0.1 mg/kg verapamil raised the heart rate. QT_c duration was not affected by 0.1 and 0.3 mg/kg verapamil (Fig. 1). The vehicle solvents did not influence the parameters of the controls.

3.2. Effects of vehicle, dofetilide, sotalol and verapamil on QT time variability

3.2.1. Poincaré plots

Before and 10 min after start of the infusions of vehicle solution or drugs, 100 consecutive ECG cycles per dog were analysed. The QT duration of a cycle was plotted against the QT duration of the subsequent cycle. Some examples are shown in Fig. 2. The plots show QT duration variability at 10 min after start of the infusions in the same individual dog that was treated on different days with vehicle, dofetilide (0.03 mg/kg), sotalol (10.0 mg/kg) and verapamil (0.3 mg/kg). Although in that particular dog P_{length} of 11.6 ms was unusually high after infusion of vehicle, the values of P_{width} confirm the visual impression of a higher beat-to-beat QT variability under treatment with dofetilide and sotalol. The Poincaré plot in this dog demonstrated a very low QT variability following infusion with verapamil.

3.2.2. Comparison of QT duration variability between treatment groups

The mean±S.E.M. values (n=6) of the parameters of QT duration variability, i.e. P_{length} and P_{width}, at baseline and at 10 min post-treatment time for the different treatment groups are shown in Table 2. There were three different control groups treated with the different solvents for dofetilide, sotalol or verapamil. Since these solvents did not have any effect on QT time variability, only one control group is shown in Table 2 for the sake of clarity.

In vehicle controls there was neither a change versus baseline of P_{width} nor P_{length}. Infusion of 0.01 and 0.03 mg/kg dofetilide increased the QT duration variability at post-treatment time (10 min p. appl.). This effect reached statistical significance for P_{length} at the high dose of dofetilide only. QT duration variability was not influenced by 3.0 mg/kg sotalol. At the higher dose of 10.0 mg/kg, sotalol significantly increased P_{length}, whereas P_{width} was increased insignificantly. Infusion of 0.1 and 0.3 mg/kg verapamil had no effect on QT time variability.

4. Discussion

QT time prolongation by itself does not correlate with the incidence of drug-induced arrhythmias. Thus, there is a need for additional predictors for the preclinical assessment of the pro-arrhythmic risk of HERG blocking agents (Belardinelli et al., 2003). In our study, we evaluated the effects of three HERG blocking agents on a new in vivo experimental parameter of cardiac repolarisation instability. In telemetered conscious dogs, the beat-to-beat QT time variability was scrutinized as a tool to discriminate agents with different pro-arrhythmic potentials. In vehicle-treated dogs QT duration variability did not change from baseline to post-treatment time, indicating the stability of this parameter under control conditions.

QT length variability was increased by dofetilide and sotalol. Both agents are considered to be pro-arrhythmic in humans. The results of our study with verapamil, which is believed to have no, or only a very low pro-arrhythmic potential, are quite different from the effects of dofetilide and sotalol. Neither the lower nor the higher dose of verapamil influenced the QT duration variability in dogs.

Dofetilide is a class III anti-arrhythmic agent (classification according to Vaughan Williams) for the treatment of atrial fibrillation (Elming et al., 2003). It blocks the

Table 2
QT duration variability in 6 serial dogs (data as mean±S.E.M.)

Treatment	Dose [mg/kg]	P_{width} [ms]			P_{length} [ms]		
		Baseline	10 min	% of baseline	Baseline	10 min	% of baseline
Vehicle	–	5.5±1.2	4.4±0.9	80	6.2±1.3	6.6±1.5	107
Dofetilide	0.01	6.4±2.0	10.9±2.5	170	8.2±2.5	11.6±2.4	142
	0.03	6.5±1.5	10.4±1.6	160	7.4±1.8	12.1±1.9*	164
Sotalol	3.0	6.5±2.5	5.0±0.7	77	5.0±1.2	6.3±1.1	126
	10.0	6.2±1.1	7.6±1.4	123	7.0±1.3	9.3±1.8*	133
Verapamil	0.1	3.4±0.6	3.0±0.4	88	5.0±0.5	4.5±0.3	90
	0.3	3.7±1.1	3.9±0.8	105	5.3±1.5	5.2±0.7	98

* p<0.05 versus baseline.

HERG encoded repolarising cardiac potassium current I_{Kr} in HEK 293 cells very potently with an IC_{50} value of 12 nM (Snyders and Chaudhary, 1996). Besides its anti-arrhythmic activity, dofetilide is potentially pro-arrhythmic in dog models (Van Opstal et al., 2001; Bauer et al., 2002) and in humans in whom it can induce TdP arrhythmias (Haverkamp et al., 2000). In dogs with chronic atrioventricular block bradycardia which are prone to TdP arrhythmias, dofetilide increased monophasic action potential duration, QT time and interventricular dispersion of QT time at a dose of 0.025 mg/kg (Van Opstal et al., 2001). A dose of 0.03 mg/kg dofetilide induced TdPs in one of six normal dogs only, but in dogs with chronic cardiac hypertrophy this dose of dofetilide induced TdPs in all five animals (Kozhevnikov et al., 2002). In our study in normal dogs, dofetilide at similar doses (0.01 and 0.03 mg/kg) not only induced a dose-dependent increase in QT_c durations but also increased QT time variability. For example, this is shown in the Poincaré plot of Fig. 2 for a dog treated with the higher dose of dofetilide (0.03 mg/kg). Compared to the vehicle group, the plot indicates a larger interbeat variability following treatment with dofetilide, suggesting a certain instability of the beat-to-beat QT time values. This visual impression could be confirmed, at least for the higher dose and for P_{length}, in a statistically significant manner. The failure of statistical significance for the lower dose and for P_{width} values most probably is related to a high deviation of the individual results (Table 2).

Sotalol is a class III anti-arrhythmic agent with an additional beta-adrenergic blocking effect (Buck et al., 1981) that mainly resides in the levorotatory isomer (Lynch et al., 1984). Sotalol inhibits the HERG potassium channel current by 80% at a concentration of 300 μM (Numaguchi et al., 2000). Inhibition of cardiac repolarisation by sotalol has been demonstrated in canine Purkinje fibres as a concentration-dependent prolongation of the action potential duration (Gintant et al., 2001) or as prolongation of left ventricular epicardial monophasic action potential duration in anaesthetised dogs (Taggart et al., 1984). Sotalol exhibits pro-arrhythmic activity including the induction of TdPs in atrioventricular blocked dogs with bradycardia and hypokalaemia (Weissenburger et al., 1991). Recently, sotalol was shown to increase short-term variability of the left ventricular monophasic action potential duration in anaesthetized dogs with electrically remodelled hearts. This increase in repolarisation variability was associated with TdP occurrence (Thomsen et al., 2004). In left ventricular wedge preparations, sotalol prolonged the action potential duration to a higher degree in mid-myocardial (M) cells than in epicardial and endocardial cells. Thus, sotalol increased the spatial heterogeneity of repolarisation, i.e. it caused different action potential durations across the ventricular wall (Shimizu et al., 1999). Infusion of 3.0 and 10.0 mg/kg sotalol dose-dependently prolonged QT_c duration in our study (Fig. 1). The scatter of the exemplary Poincaré plot (Fig. 2) also indicates a high degree of variability of the QT duration values from one beat to the next in a dog treated with 10.0 mg/kg sotalol. The QT duration variability was increased by sotalol as indicated by the significant change of P_{length} versus baseline at the higher dose (Table 2).

Verapamil is a phenylalkylamine calcium channel blocker used on a broad clinical scale. The activity of verapamil is not restricted to calcium channels. It inhibits various potassium channels involved in cardiac repolarisation with different potencies. HERG channel activity (Zhang et al., 1999) and I_{Kr} current in guinea pig atrial myocytes (Jones et al., 2000) are inhibited by verapamil with IC_{50} values of 0.143 μM and 3 μM, respectively. Although verapamil interferes with cardiac repolarising potassium channels, it has a low propensity to prolong QT time and there is no evidence that it induces TdP arrhythmias (Redfern et al., 2003). Verapamil even reverses the action potential duration prolonging activity of the HERG blocker E-4031 (1-[2(6-methyl-2-pyridyl)ethyl]-4(4-methylsulfonylamidobenzoyl) piperidine) in guinea pig papillary muscles (Bril et al., 1998). In contrast to pro-arrhythmic HERG channel blockers, verapamil was shown not to increase beat-to-beat alternations of the cardiac monophasic action potential in guinea pigs (Fossa et al., 2004). This favourable safety profile of verapamil regarding QT time prolongation and related arrhythmias is considered to be the result of a suitable balance of potassium and calcium channel blocking activities. In our study, at both doses (0.1 and 0.3 mg/kg), verapamil did not change the QT_c duration (Fig. 1). The scatter of an exemplary Poincaré plot is low for a dog treated with 0.3 mg/kg verapamil (Fig. 2). Furthermore, verapamil did not influence QT time variability at any dose (Table 2). This is in line with other reports that suggest a low pro-arrhythmic potential of verapamil.

It might be argued that changes in heart rate may account for the QT duration variability observed after infusion of pro-arrhythmic HERG channel blockers. QT times adapt to changes in heart rate but the changes in QT time lag somewhat behind the changes in heart rate (Lau et al., 1988). This hysteresis effect may be responsible for the greater variation of the QT length and thus indicate a false positive effect on repolarisation instability. However, a comparison of the heart rate and QT duration values in dogs treated with dofetilide or sotalol show that 10 min after start of the infusions (the time when the beat-to-beat analysis was performed), no abrupt changes in heart rate occurred (Fig. 1). On the other hand, heart rate fell considerably 5–10 min after start of the infusion of 0.3 mg/kg verapamil, but the QT length variability did not change at all in this experimental group. Thus, the observed effects on QT length variability are independent of changes in heart rate.

The probability that a HERG blocker will induce arrhythmias is related to its ability to induce dispersion or

to increase heterogeneity of ventricular repolarisation. Spatial heterogeneity of ventricular repolarisation can be evaluated as transmural dispersion across the left ventricular wall (Milberg et al., 2002; Antzelevitch and Shimizu, 2002), between the right and left ventricles (Schoenmakers et al., 2003; Van Opstal et al., 2001) or between the base and the apex of the heart (Bauer et al., 2002). Not only spatial heterogeneity but also temporal heterogeneity of repolarisation may indicate an enhanced risk of pro-arrhythmia. Temporal heterogeneity, demonstrated as beat-to-beat variations in action potential duration in isolated rabbit hearts, signalised repolarisation instability and was found to be a reliable predictor of the pro-arrhythmic risk of HERG channel blocking agents (Hondeghem et al., 2001, 2003; Hondeghem and Hoffmann, 2003). Recently, a differentiation of pro-arrhythmia and non-arrhythmic HERG channel blockers was shown in a model of cardiac electric alternans in the guinea pig (Fossa et al., 2004). These models demonstrated the induction of increased temporal heterogeneity of cardiac repolarisation by pro-arrhythmic HERG channel blockers.

Similar to these models, our study in conscious dogs was performed on the same assumption that pro-arrhythmia agents increase temporal dispersion of cardiac repolarisation whereas non-arrhythmic agents do not. The results obtained with the HERG channel blockers dofetilide, sotalol and verapamil indicate that the parameter of QT time variability can discriminate between agents with a high and low risk of inducing arrhythmias. HERG blockers which are associated with arrhythmias of the TdP type as dofetilide and sotalol increased the QT time variability. On the other hand, verapamil as a HERG blocker which is not associated with TdP did not change the QT duration variability in conscious dogs.

The increases of QT duration variability by dofetilide and sotalol reached statistical significance only on the parameter P_{length} but not P_{width}. Although a clear trend to an increase was also seen for P_{width} at both doses of dofetilide and at the high dose of sotalol, statistical significance was not reached most probably because group size was too low. Therefore, further studies including these parameters of QT duration variability should use larger group sizes.

The model of QT duration variability is based on studies in conscious dogs which are not surgically prepared for the induction of heart disease. The native status of the animals used in this study may explain that the pro-arrhythmogenicity of agents such as dofetilide and sotalol does not lead to the occurrence of arrhythmias. The QT duration variability is thought to be a surrogate parameter of the temporal heterogeneity of cardiac repolarisation that indicates an enhanced risk of pro-arrhythmia. The model does not allow any statement on the influence of agents on the spatial heterogeneity of cardiac repolarisation. Despite these limitations, the measurement of the QT duration variability in conscious dogs may provide further valuable information in the preclinical risk assessment of HERG blocking agents.

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