Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 554 (2007) 46–52
www.elsevier.com/locate/ejphar

Simple-to-use, reference criteria for revealing drug-induced QT interval prolongation in conscious dogs

Michael W. Schmitt *, Friedrich Von Landenberg, Hartmut Poth, Elmar Wimmer, Thomas Goddemeier, Icilio Cavero [1]

MerckKGaA, Pharma Ethicals, Research and Development, D-64271 Darmstadt, Germany

Received 17 May 2006; received in revised form 19 September 2006; accepted 22 September 2006
Available online 10 October 2006

Abstract

Electrocardiogram (ECG) QT interval prolongation produced by drugs in certain animal models is currently believed to be predictive of cardiac proarrhythmic effects in humans. For this reason, nonclinical assessment of the effects of novel drugs on cardiac repolarization is a regulatory prerequisite for progressing such agents to clinical evaluation. The present investigation was carried out to develop reliable, simple-to-use reference criteria for identifying individual animals as responders to drugs that prolong the QT interval. ECG were recorded for 30 s at 0 (8 am), 2, 4, 6 and 24 h in 6 trained, conscious, beagle dogs during 5 control experimental sessions. QT intervals were measured and corrected for heart rate by applying the Van de Water algorithm (QTc). The maximal (QTc_{max}) and minimal (QTc_{min}) values of QTc observed in each of the five control recording sessions were noted. Two reference (R) criteria were used to designate an individual animal as a responder to drug treatment: 1) QTc_{maxR} which was obtained by adding 10 ms to the largest value of QTc_{max} observed during the five control recording sessions and 2) $(QTc_{max}-QTc_{min})_{maxR}$ which was obtained by increasing by 50% the largest of the $(QTc_{max}-QTc_{min})$ values $[(QTc_{max}-QTc_{min})_{max}]$ observed in the 5 control recording sessions. The sensitivity and reliability of these criteria were tested by determining QTc intervals before and 2, 4, 6 and 24 h after placebo or quinidine (200, 400 and 800 mg p.o. per animal). The reference values of QTc_{maxR} and $(QTc_{max}-QTc_{min})_{maxR}$ for the various dogs ranged from 246 to 270 ms and from 15 to 19.5 ms, respectively. The number of dogs responding to treatment (T: quinidine at 200, 400 and 800 mg, p.o. per animal) with a QTc_{maxT} and/or a $(QTc_{max}-QTc_{min})_{maxT}$ equal to or greater than the respective reference values was, respectively, 1/6, 3/6 and 5/6 dogs. Additionally, the number of responders correlated well with the concentration of free quinidine in the plasma. In conclusion, this investigation succeeded in establishing reliable, reference criteria for individual dogs despite the intrinsic daily variation of QTc interval. The application of these criteria allowed identifying individual animals responding to quinidine with delayed cardiac repolarization.

Keywords: ECG; Trained conscious dog; Quinidine; QT interval; Reference criteria for QT interval prolongation

1. Introduction

Repolarization is a vital electrophysiological process responsible for returning activated cardiac myocytes to a resting state. Drugs prolonging this critical phase of the cardiac cycle have an inherent tendency to trigger proarrhythmic episodes, particularly in patients with cardiac diseases that can critically reduce the native repolarization reserve that protects the healthy heart against proarrhythmic insults (Roden, 2006).

In 2005, the International Conference on Harmonization (ICH) approved the S7B guideline, outlining a preclinical testing strategy for possible cardiac repolarization problems induced by drugs (ICH, 2005). The experimental strategy advocated by the document comprises two core tests. The first involves the *in vitro* measurement of the effects of novel compounds on the rapid cardiac delayed rectifier current in cloned *h*ERG (human ether-a-go-go-related gene) channels. These channels play an antifibrillatory role during the repolarization process of the human heart. The second test involves *in vivo* recording of the electrocardiogram, usually in the conscious dog. The predictive power of these tests for the clinical outcome measured as prolongation of QTc by more than 5 ms (the threshold level for regulatory concern) is still a matter of active debate. Indeed, in a

* Corresponding author. MerckKGaA, Pha Preclinical R&D, Global Toxicology, Frankfurter Str. 250, 64293 Darmstadt, Germany. Tel.: +49 6151 72 8976; fax: +49 6151 72 7673.
E-mail address: Michael.w.schmitt@merck.de (M.W. Schmitt).
[1] Via dei Bacchettoni, 19B 55100 Lucca, Italy.

0014-2999/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2006.09.056

recent FDA (Food and Drug Administration) analysis, 10 out of 19 drugs analyzed prolonged QTc interval ≥5 ms in healthy volunteers. Of these 10 drugs, 5 and 4 drugs were, respectively, found positive in the *in vitro h*ERG channel, and the *in vivo* QTc interval assay with only 1 being positive in both tests. An additional problematic outcome of this analysis was that 2 of the 10 clinically positive drugs were not detected by the strategy outlined in the S7B guidelines (Cavero and Crumb, 2005b, 2006). The poor predictive power of these preclinical tests was likely due, at least in part, to the experimental protocols and conditions, which are rarely optimized to maximize sensitivity and minimize variability (Cavero and Crumb, 2005a,b, 2006). Thus, it is of central importance to any safety investigation to adopt experimental protocols that can reliably detect the propensity of a drug adversely to affect cardiac repolarization (Cavero and Crumb, 2006).

The present investigation was conducted in order to identify simple, easy-to-use criteria that might allow the classification of individual animals, as responders to novel drugs with the potential to prolong the QT interval. Its aim was to facilitate selection of the safest members from a group of novel drugs developed for a particular clinical application. The usefulness and sensitivity of the proposed criteria was tested by evaluating the repolarization effects of quinidine, a drug known to prolong the QT interval in animals (Olivier et al., 2003; Testai et al., 2004) and humans (Cubeddu, 2003; Malik and Camm, 2001).

2. Materials and methods

Local health authorities were informed of the experimentation described in this study. Animal handling was in accordance with German Law on the protection of animals (Article 8).

2.1. Drugs

Commercially available tablets (Chinidin-Duriles®) containing quinidine hydrogen sulfate 4 H_2O (corresponding to 200 mg quinidine) were placed in hard gelatin capsules for oral administration. Empty capsules were used for placebo treatment.

2.2. Animals and training

Six (3 male/3 female) trained beagle dogs (Harlan-Winkelmann GmbH, Borchen), 14 and 15 months old and weighing 9–14 kg at the beginning of the study, were identified by tattoos in their right ears. They were housed singly in pens that were divided into indoor and outdoor (runs) areas of similar surface (~3.3 m^2).

Acclimation of the dogs to the laboratory environment was initially achieved by training the animals to lie in a thoracic-abdominal position during 8 sessions lasting 1 h/day before being used for the first control study.

2.3. Examinations

Behaviour, general condition, and food consumption were assessed daily for each study subject. Body weights were measured at the onset, and weekly throughout the study. Serum chemisty and hematology were performed before each study in order to verify the health of the animals. Only animals with normal pre-study evaluations were included in the protocol.

2.4. Administration method and quinidine dose levels

One, 2 or 4 quinidine tablets were placed in a hard gelatin capsule for the oral administration of 200, 400 and 800 mg of quinidine per animal under fasting conditions at ~8 am. The animals were generally fed between 8.45 and 10.45 am. Quinidine doses were not standardized to the animal weight, thereby mimicking the majority of orally-administered drug therapies in humans. A washout period of at least 2 to 5 days between successive treatments was observed. The overall duration of the placebo/quinidine investigation was 17 days.

2.5. ECG measurements

Using classic (I, II, and III) and unipolar (aVR, aVL, aVF) leads, ECGs were recorded (Hellige EK 53) from animals in thoracic-abdominal recumbancy for a 30 s period at ~8 am and 2, 4, 6 and 24 h during 5 control sessions. Similarly, recordings were made in 4 additional sessions during which the animals received one of the 3 different doses of quinidine, or placebo. The recumbent position yielded noise-free ECG tracings, minimizing the variation in individual ECG parameters between the various test sessions. No electronic filters were used during ECG signal acquisition. ECGs were recorded at 50 mm/s on a chart recorder calibrated to display ECG signals at 1 cm/mV. Using 20 s ECG segments, two independent experts in ECG assessment performed independent measurements of heart rate (beats/min), PQ, QRS and QT intervals. Measurements were made using an ECG ruler applied to the 10 best noise-free ECG complexes within the 20 s sample. The QT interval was defined as the time from the onset of the Q-wave to the point at which the downslope of the T-wave intersected the ECG isoelectric baseline. The duration of QT interval was measured to the nearest 10 ms unit on the ECG ruler. The values from the 10 ECG complexes were then averaged for greater accuracy although Hamlin et al. (2004) have reported that QT measurements from a single cardiac cycle accurately reflect QT intervals averaged from 3, 6, or 12 successive cardiac cycles.

2.6. Algorithm to correct QT for heart rate

QT intervals measured on each ECG lead were corrected according to Van de Water's (QTc) algorithm $QTc = QT - 0.087 \times [(60/HR) - 1]$ (Van de Water et al., 1989), where HR indicates heart rate.

2.7. Determination of reference QTc criteria

Data for the determination of the variability of QTc interval (~8 am–~2 pm) was generated in 5 independent, treatment-free experimental sessions performed in the same group of 6 trained conscious dogs over a 4 year period. During this period, the same animals were also used to study the effects of active

Table 1
A representative dataset from 1/6 dogs studied in this investigation

Male dog N° 683	QTc (ms)								
	Experimental session								
	C_1	C_2	C_3	C_4	C_5	Q_0	Q_{200}	Q_{400}	Q_{800}
Sampling time									
0 h	240	236	240	239	236	240	233	240	231
2 h	245	240	237	239	244	245	247	247	246
4 h	241	237	237	239	241	241	245	242	257
6 h	240	234	241	241	241	240	240	248	260
24 h	237	233	234	237	234	237	234	233	236
QTc_{min} (ms)	237	233	234	237	234	237	233	233	231
QTc_{max} (ms)	245	240	241	241	244	245	247	248	260
(QTc_{max}-QTc_{min} (ms)	8	7	7	4	10	8	14	15	29

The table presents QTc values generated during 5 control sessions (C_1–C_5) and after the oral administration of quinidine: 0 (placebo: Q_0), 200 (Q_{200}), 400 (Q_{400}), and 800 (Q_{800}) mg/animal.
QTc_{min} and QTc_{max} values and the difference ($QTc_{max}-QTc_{min}$) are shown.
The shaded cells identify the largest individual control QTc_{max} (245 ms) and the largest $(QTc_{max}-QTc_{min})_{max}$ (10 ms) values observed in the 5 control sessions.

treatments, allowing at least a 4–8 week washout period between consecutive treatments.

The largest individual QTc_{max} for each animal obtained during the five treatment-free sessions was identified (Table 1, columns 1–5). The largest individual difference $(QTc_{max}-QTc_{min})_{max}$ observed during the 5 treatment-free sessions was then calculated (Table 1).

Two reference (R) criteria were used to identify individual animals as responders to a given treatment: 1) QTc_{maxR} — obtained by adding 10 ms to the largest value of QTc_{max} observed during the 5 treatment-free sessions, and 2) $(QTc_{max}-QTc_{min})_{maxR}$ which is defined as the largest value of $(QTc_{max}-QTc_{min})$ observed during the 5 control sessions, and increased by 50% (Tables 1 and 2).

2.8. Statistical analysis

Data are reported as individual observations or as means ± S.D. For each experimental session, individual QTc_{max} and QTc_{min} were identified and the difference $(QTc_{max}-QTc_{min})_{max}$ calculated (Table 1). An analysis of variance (ANOVA) was performed to identify quinidine-induced QT interval changes. Comparisons of differences between mean values were performed employing a Tukey–Kramer test. QTc interval effects are reported as adjusted mean values (LSMeans adjusted for factors of ANOVA-model).

Table 2
Individual reference (R) criteria QTc_{maxR} (=QTc_{max} + 10) and $(QTc_{max}-QTc_{min})_R$ [=$(QTc_{max}-QTc_{min})_{max}$ increased by 50%] determined from the dataset generated with 6 dogs subjected to 5 treatment-free sessions

Dog N°	Sex	QTc_{maxR} (ms)	$(QTc_{max}-QTc_{min})_{IR}$ (ms)
651	M	270	19.5
662	M	258	16.5
683	M	255	15.0
670	F	246	16.5
676	F	256	16.5
677	F	255	18.0

2.9. Pharmacokinetics

Venous blood samples for the determination of quinidine pharmacokinetics were obtained at 0, 2, 4, 6, and 24 h after the administration of each dose, and immediately after each ECG recording. The analytical detection limit was 25 ng quinidine/mL of plasma. Pharmacokinetic parameters included peak plasma concentration (C_{max}) and time at which the peak plasma concentration occurred (T_{max}). The area under the plasma concentration/time (0–24 h) curve ($AUC_{0\rightarrow 24\ h}$) was calculated using the trapezoidal rule.

3. Results

3.1. Determination of parameters describing QT interval

The present dataset was generated by determining QT intervals from classic ECG leads in 6 trained conscious dogs throughout the day at 0 (8 am) and 2, 4, 6 and 24 h during 5 treatment-free and 4 drug (quinidine) treatment sessions.

QTc values were virtually identical, regardless of which ECG lead was selected for QT interval measurement (data not reported). Accordingly, ECG limb-lead II was employed for the current QT interval determinations. Independent measurements performed by two ECG experts did not differ significantly.

A representative dataset for 1 of the 6 dogs used in this study is shown in Table 1. The data in columns headed C_1–C_5 were generated during 5 control experimental sessions whereas data in columns headed Q_0, Q_{200}, Q_{400} and Q_{800} are those gathered after administration of quinidine at 0 (placebo), 200, 400, and 800 mg/animal. For each session and for each animal, QTc minima (QTc_{min}) and QTc maxima (QTc_{max}) were identified and the difference ($QTc_{max}-QTc_{min}$) calculated. For each animal, the largest QTc_{max} and the largest value of ($QTc_{max}-QTc_{min}$) among the 5 treatment-free sessions were then identified (shaded boxes in Table 1) for establishing reference criteria (shaded boxes in Table 1).

Individual QTc_{min} and QTc_{max} values varied between 233 to 253 and 236 to 260 ms, respectively, during the 5 control sessions (experiment numbers C_1–C_5 and Q_0 in Table 1) for all studied animals while the $(QTc_{max}-QTc_{min})_{max}$ varied between 4 to 13 ms. The within-animal variability for QTc_{min} and QTc_{max} was 4–8% whereas the within-animal variability of $(QTc_{max}-QTc_{min})_{max}$ was 40–70%.

Table 3
Pharmacokinetic data (mean ± S.D.) for dogs (3 males and 3 females) treated with 3 doses of quinidine per os. C_{max} was attained between 4 and 6 h after administration

Dose (mg/animal)	Body weight (kg)	C_{max} (ng/mL)	T_{max} (h)	$C_{24\ h}$ (mg/mL)	$AUC_{0\rightarrow 24\ h}$ (ng/mL*h)
200	12±2	1945±55	4–6	131±47	19,800±800
400	13±2	2970±240	4–6	649±252	39,050±6150
800	12±2	4180±70	4–6	1470±170	63,350±3650

3.2. Reference criteria for determining treatment-induced QTc prolongation

Two individual reference (R) criteria, to be separately or concurrently satisfied, were identified to identify any given animal as a QTc positive responder. The first individual reference criterion (QTc_{maxR}) was taken to be the largest individual QTc_{max} among those measured during the 5 treatment-free sessions, incremented by 10 ms (Table 2). The second criterion [$(QTc_{max} - QTc_{min})_{maxR}$] was defined as the largest individual value of ($QTc_{max} - QTc_{min}$) obtained during the 5 treatment-free sessions, incremented by 50% (Table 2).

3.3. Study of quinidine

3.3.1. Pharmacokinetics

The maximal concentration of free quinidine in the plasma (C_{max}) and the area under the plasma concentration/time curve ($AUC_{0\rightarrow 24\ h}$) increased as function of the quinidine dose (Table 3). The time for quinidine to attain a peak plasma concentration (T_{max}) was between 4 and 6 h after administration. In 5 of 6 animals, quinidine was still detectable in the plasma 24 h after dose (Table 3).

3.3.2. Clinical effects

All animals receiving the highest dose of quinidine (800 mg/animal) vomited within 2 to 4 h after dosing. Although no drug or capsule residues were noticed in the vomitus, this effect may have reduced C_{max} and $AUC_{0\rightarrow 24\ h}$ (Table 3). Two dogs displayed photophobia starting 2 h after receiving the highest dose, and persisting for approximately 4 h. No notable clinical symptoms were observed in animals treated with either the 200 or 400 mg doses of quinidine.

3.3.3. Heart rate effects

Baseline heart rates prior to placebo administration (108±32 beats/min) and prior to each of the 3 quinidine doses were virtually identical (101±20; 103±20; 107±11 beats/min, for 200, 400, and 800 mg, respectively). Heart rates, expressed as the change from baseline were 4±6, 30±7, and, 30±14 beats/min 4–6 h T_{max}) after quinidine dosing. As shown, heart rate changes following placebo administration were very similar (25±7 beats/min) to those measured after the two higher doses of quinidine while the change following the lowest dose of quinidine was less than that observed with placebo treatment.

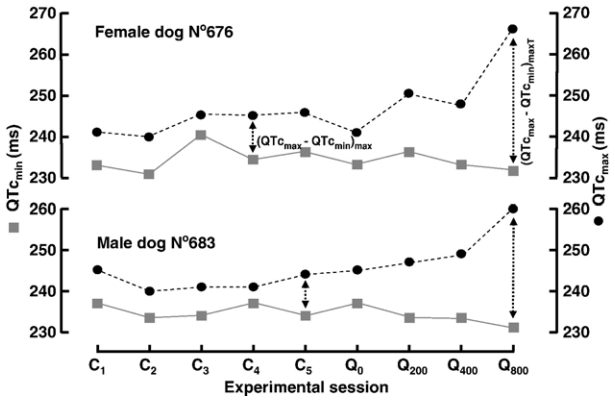

Fig. 1. Minimal (QTc_{min}) and maximal (QTc_{max}) values of QTc measured in 2/6 dogs used in this investigation. The illustrated data were generated in five treatment-free sessions (C1–C5) and after 200 (Q_{200}), 400 (Q_{400}) and 800 (Q_{800}) mg/animal of orally administered quinidine or placebo (Q_0). The maximal difference [$(QTc_{max} - QTc_{min})_{max}$] observed in the 5 control sessions (C_1–C_5) and the [$(QTc_{max} - QTc_{min})_{maxT}$ after 800 mg quinidine (Q_{800} session) are indicated by double-ended arrows.

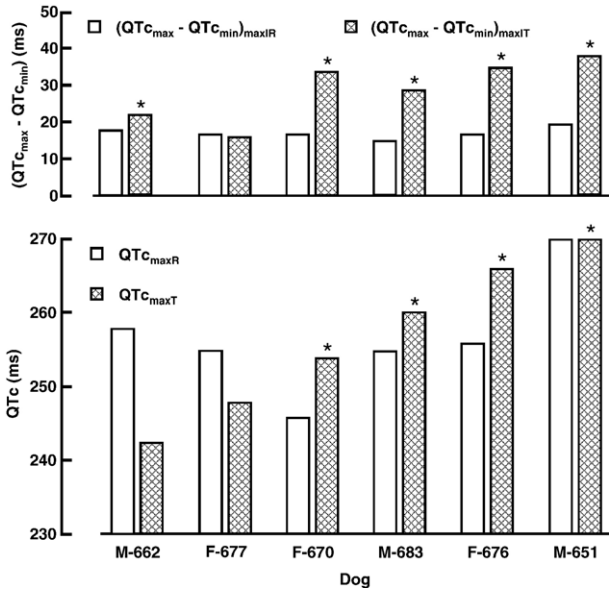

Fig. 2. Values of reference criteria [QTc_{maxR} and $(QTc_{max} - QTc_{min})_{maxR}$] for individual dogs and values [QTc_{maxT}, $(QTc_{max} - QTc_{min})_{maxT}$] following the oral administration of quinidine (800 mg/animal) to 3 male (M) and 3 female (F) dogs. QTc_{maxR} was obtained by adding 10 ms to the largest individual QTc_{max} observed in 5 control sessions whereas $(QTc_{max} - QTc_{min})_{maxR}$ was taken as the largest difference $(QTc_{max} - QTc_{min})_{max}$ observed for the 5 treatment-free sessions augmented by 50% (see Table 1 and Fig. 1). An asterisk indicates that the response after quinidine was identical to, or exceeded, the respective reference criteria value.

3.3.4. QTc interval effects

Administration of placebo (empty capsules) elicited QT interval changes that were similar to those obtained during five

Table 4
Dogs judged to be responders (indicated with an X) on the basis of QTc_{maxR} (CR_1) and/or $(QTc_{max} - QTc_{min})_R$ (CR_2) reference criteria after treatment with 200 (Q_{200}), 400 (Q_{400}), and 800 (Q_{800}) mg per os quinidine

Dog N°	QTc interval positive responder (X)					
	Q_{200}		Q_{400}		Q_{800}	
	CR_1	CR_2	CR_1	CR_2	CR_1	CR_2
651				X	X	X
662						
683				X	X	X
670		X		X	X	X
676					X	X
677						X

The shaded cells indicated animal responding to a single criterion after the studied dose of quinidine.

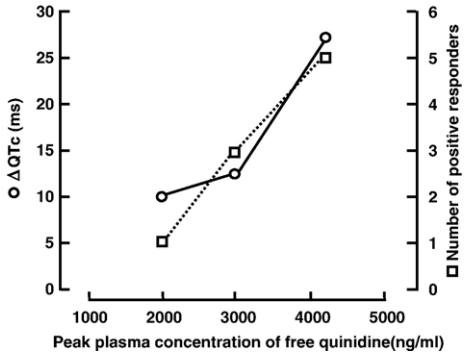

Fig. 3. Mean increases in QTc as a function of the peak plasma concentration of free quinidine in trained, conscious dogs ($n=6$). The number of dogs responding with values equal to, or larger than, those set for the reference criteria [QTc_{maxR} or $(QTc_{max}-QTc_{min})_{maxR}$] (see Fig. 2 for results with the highest dose of quinidine used) are also plotted against the respective peak plasma concentrations of free quinidine.

control experimental sessions (Table 1 and Fig. 1). However, QTc_{maxT} for dogs 651, 670, 676 and 683, treated with 800 mg quinidine (Fig. 2) attained values exceeding the respective reference criteria (Tables 2 and 4). As shown in Fig. 2, individual animals where $(QTc_{max}-QTc_{min})_{maxT}$ exceeded the respective reference values (Table 2) were dog 670 (200 mg), dogs 651, 670, 683 (400 mg), and dogs 651, 670, 676, 677 and 683 (800 mg) (Fig. 2, Table 4). After dosing with 200, 400 and 800 mg quinidine, 1/6, 3/6 and 1/6 animals respectively were judged to be responders on the basis of the $(QTc_{max}-QTc_{min})_{maxT}$ criterion alone. For each of the doses of quinidine used, all animals judged to be responders on the basis of QTc_{maxT} were also judged to be responders on the basis of the criterion $(QTc_{max}-QTc_{min})_{maxT}$. At none of the tested doses of quinidine was dog 662 judged to be a responder by either of the two criteria (Table 4)

The QTc_{maxT} and $(QTc_{max}-QTc_{min})_{maxT}$ effects occurred at the time of the peak plasma concentration of free quinidine. The number of animals responding with QTc interval prolongation was directly proportional to the quinidine free plasma concentration (Fig. 3). Quinidine also produced significant group mean increases (ANOVA) in the QTc interval. These were also directly related to the dose of quinidine and the associated free plasma concentrations (Fig. 3).

4. Discussion

Identifying and quantifying the potential of novel drugs to delay cardiac repolarization plays a major role in the preclinical and clinical development of medicines for human use, and is intended to eliminate those compounds that carry a risk for promoting life-threatening cardiac arrhythmias such as TdP. Failure to assess this risk appropriately may delay, and even prevent, the granting of marketing approval for a drug.

The key objective of this investigation was to establish reliable reference criteria to correctly categorize individual experimental subjects as positive responders to a drug known to prolong the cardiac QT interval. The first criterion was QTc_{maxR} which represents the largest value of QTc_{max} observed during 5 control ECG sessions increased by 10 ms. The second reference criterion, $(QTc_{max}-QTc_{min})_{maxR}$, was defined as the largest value of $(QTc_{max}-QTc_{min})$ observed during 5 control ECG sessions, increased by 50%. Our decision to increase the experimentally determined values of QTc_{max} and $(QTc_{max}-QTc_{min})_{max}$ by 10 ms and 50%, respectively, was designed to establish a reliable threshold that would clearly rule out a risk of delayed cardiac repolarization in the event that none of the drug-treated animals was a positive responder. However, the latter increasing factors should be considered for guidance only, and may be adjusted to modulate the sensitivity and reliability of the test within each laboratory. In our experience, the current reference criteria clearly identified drugs known to prolong the cardiac QT interval. These included quinidine (data reported herein), dofetilide, and sotalol.

Selecting QTc_{maxR} as a reference criterion was an intuitive process since any increase in this parameter reflects a reduced repolarization reserve. In contrast, the criterion $(QTc_{max}-QTc_{min})$ was developed as result of a careful analysis of data generated in our laboratory. These results indicated that treatment with drugs, which are known to prolong the QT interval (e.g. quinidine, dofetilide and sotalol), increased this parameter compared with control sessions, as clearly illustrated in Figs. 1 and 2, and Table 4. These increases were almost exclusively due to increases in QTc_{max} as QTc_{min} during drug treatment sessions did not differ substantially from those values measured before or 24 h after quinidine administration. The $(QTc_{max}-QTc_{min})_{maxR}$ criterion appears to be more sensitive than QTc_{maxR} for the detection of positive quinidine responders as 5/6 dogs were positive based on this criterion, whereas only 1/6 dogs were positive based upon QTc_{maxR} (Table 4). While of differing sensitivities, both of the current reference criteria are likely to reflect drug-induced slowing of ventricular repolarization, represented by the T-wave of the electrocardiogram. Such an effect is currently regarded as predictive of proarrhythmic incidents in patients with poor cardiac repolarization reserve (Antzelevitch, 2005; Antzelevitch and Oliva, 2006; Roden, 2006).

In our laboratory, an animal is considered as a positive QT interval responder if, the values of QTc_{maxT} or $(QTc_{max}-QTc_{min})_{maxT}$ match or exceed the values of the respective reference criteria after drug treatment (T). The validity of this approach was confirmed by studying quinidine, a drug known to prolong the QT interval in animals (Olivier et al., 2003; Testai et al., 2004) and humans (Cubeddu, 2003; Malik and Camm, 2001). Of the 6 animals treated with the highest dose of quinidine (800 mg total), four responded with a QTc_{maxT}, and five with a $(QTc_{max}-QTc_{min})_{maxT}$, that were identical to, or exceeded, the respective reference values. Three of 6 animals treated with the middle dose, and 1/6 dogs treated with the lowest dose were classified as QTc responders according to our criteria. In all cases, the number of responders was well correlated with the free quinidine plasma concentrations. With placebo-treatment (P), the values of QTc_{maxP} or $(QTc_{max}-QTc_{min})_{maxP}$ never matched or exceeded either of the established reference criteria. This observation indicates that the

current reference criteria are of adequate sensitivity to reliably detect drug-induced changes in the QT interval. Interestingly, the single dog identified as a positive responder following treatment with the low dose of quinidine had a peak plasma concentration of free quinidine of 1620 ng/mL. This value is close to the plasma concentrations of quinidine affording therapeutic activity (2000–5000 ng/mL) and concurrently increasing the QT interval in humans (Roden, 1991; Goodman and Gilman, 1995). Quinidine-induced *torsades de pointes* have been observed following oral doses producing both therapeutic and sub-therapeutic plasma concentrations (Roden, 1991). This indicates the important role of the cardiac state of the patient in determining QT interval prolongation and its potential proarrhythmic consequences. Our criteria appear to correctly identify dogs as positive QT interval responders at plasma concentrations of free quinidine that produce therapeutic and cardiotoxic effects in humans.

In contrast to commonly used safety pharmacology protocols, the present criteria were developed to identify individual animals within a treated group that exhibited enhanced susceptibility to agents causing prolongation of the QT interval. Individual susceptibility to drugs delaying cardiac repolarization is likely to underlie inter-individual differences in native cardiac repolarization reserve and is affected by numerous factors such as gender, plasma electrolytes, inherited, and acquired diseases (Roden, 2006).

A possible confounding factor in this investigation is the use of a predetermined algorithm (Van de Water's formula) to correct the measured QT intervals for heart rate contribution. When a treatment produces substantial changes in heart rate, QT rate-correction algorithms are not satisfactory, and may provide inaccurate QTc interval estimations (Malik, 2002). This does not appear to be the case in this study, since heart rate changes from baseline following placebo and following the two higher doses of quinidine were virtually identical. Thus, it is unlikely that the current conclusions were adversely affected by the use of the Van de Water QT rate-correction. However, cases where the test article significantly alters heart rate will require accurate QT rate-correction before application of the current reference criteria.

The appropriate assessment of cardiovascular risk is a critical issue in all safety pharmacology studies. With the current criteria, which number of positive QT interval responders provides sufficient evidence for an unacceptable risk for delayed cardiac repolarization in humans? This is a complex problem, and a definitive answer is beyond the scope of the current investigation. Criteria for the selection of drugs for clinical development are established based on multiple, and often company specific, standards.

In our institution, and using the current criteria, we generally have not selected compounds, which have yielded a single positive QT interval response at plasma concentrations foreseen for therapeutic activity. It should be noted, however, that novel drugs are characterized in the present assay studying 3 ascending doses, selected to yield therapeutic and supra-therapeutic plasma concentrations. In our experience, a dose-related increase in the number of positive QT responders, as illustrated in this report, usually identifies agents that clearly prolong the QTc interval. The timing of our *in vivo* QT safety assessment, which is implemented very early during the drug development process, necessitates this conservative drug selection policy. At this early stage, it is still possible to search for new chemical derivatives of a compound identified as QT interval positive in our model that have a superior cardiac safety profile. If such derivatives show no sign of improved cardiac safety, then the original compound may be selected for additional cardiac safety evaluation, but only if it has potential clinical advantage over existing therapies.

In conclusion, this investigation identified two novel and reliable reference criteria that may be advantageously employed to detect drug-induced delayed cardiac repolarization in individual dogs.

Acknowledgements

The authors wish to thank Drs Barbara Gottschling, Alexander Breidenbach, Henry Holzegrefe and Roger Small for their critical reading of the manuscript.

References

Antzelevitch, C., 2005. Role of transmural dispersion of repolarization in the genesis of drug-induced torsades de pointes. Heart Rhythm 2 (2 Suppl), S9–S15.

Antzelevitch, C., Oliva, A., 2006. Amplification of spatial dispersion of repolarization underlies sudden cardiac death associated with catecholaminergic polymorphic VT, long QT, short QT and Brugada syndromes. J. Intern. Med. 259, 48–58.

Cavero, I., Crumb, W., 2005a. ICH S7B guideline on the clinical strategy for testing delayed cardiac repolarization risk of drugs: a critical analysis. Expert Opin. Drug Saf. 4, 509–530.

Cavero, I., Crumb, W., 2005b. The use of electrocardiogram in clinical trials: a public discussion of the proposed ICH E14 regulatory guidance. Expert Opin. Drug Saf. 4, 795–799.

Cavero, I., Crumb, W., 2006. Moving towards better predictors of drug-induced torsade de pointes. Expert Opin. Drug Saf. 5, 335–340.

Cubeddu, L.X., 2003. QT prolongation and fatal arrhythmias: a review of clinical implications and effects of drugs. Am. J. Ther. 10, 452–457.

Goodman & Gilman's, 1995. In: Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W., Goodman Gilman, A. (Eds.), The Pharmacological Basis of Therapeutics, 9th edition. Mc Graw-Hill, New York, NY. Section V. pp. 870– ???.

Hamlin, R.L., Kijtawornrat, A., Keene, B.W., 2004. How many cardiac cycles must be measured to permit accurate RR, QT, and QTc estimates in conscious dogs? J. Pharmacol. Toxicol. Methods 50, 103–108.

ICH, 2005. The Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals (S7B). http://www.ich.org/LOB/media/ MEDIA2192.pdf.

Malik, M., 2002. The imprecision in heart rate correction may lead to artificial observations of drug induced QT interval changes. Pacing Clin. Electrophysiol. 25, 209–216.

Malik, M., Camm, A.J., 2001. Evaluation of drug-induced QT interval prolongation: implications for drug approval and labelling. Drug Safety 24, 323–351.

Olivier, N.B., Eyster, G.E., Sanders, R., Cheng, J., Bohart, G., Girand, M., Bailie, M., 2003. Atrioventricular nodal ablation and His-bundle pacing: an acute canine model for proarrhythmic risk assessment. J. Cardiovasc. Electrophysiol. 14, 1356–1360.

Roden, D.M., 1991. Quinidine. In: Dangman, K.H., Miura, D.S. (Eds.), Electrophysiology and Pharmacology of the Heart. Marcel Dekker, New York, NY, pp. 493–516.

Roden, D.M., 2006. Long QT syndrome: reduced repolarization reserve and the genetic link. J. Intern. Med. 259, 59–69.

Testai, L., Calderone, V., Salvadori, A., Breschi, M.C., Nieri, P., Martinetti, E., 2004. QT prolongation in anaesthetized guinea-pigs: an experimental approach for preliminary screening of torsadogenicity of drugs and drug candidates. Appl. Toxicol. 24, 217–222.

Van de Water, A., Verheyen, J., Xhonneux, R., Reneman, R.S., 1989. An improved method to correct the QT interval of the electrocardiogram for changes in heart rate. J. Pharmacol. Methods 22, 207–217.