

RESEARCH PAPER

Predicting drug-induced changes in QT interval and arrhythmias: QT-shortening drugs point to gaps in the ICHS7B Guidelines

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Background and purpose: The regulatory guidelines (ICHS7B) recommending inhibition of the delayed rectifier K⁺ current (I_{Kr}), carried by human ether-a-go-go-related gene (hERG) channels in cardiac cells (the hERG test), as a ‘first line’ test for identifying compounds inducing QT prolongation, have limitations, some of which are outlined here.

Experimental approach: hERG current was measured in HEK293 cells, stably transfected with hERG channels; action potential duration (APD) and arrhythmogenic effects were measured in isolated Purkinje fibres and perfused hearts from rabbits.

Key results: 576 compounds were screened in the hERG test: 58% were identified as hERG inhibitors, 39% had no effect and 3% were classified as stimulators. Of the hERG inhibitors, 92 were tested in the APD assay: 55.4% of these prolonged APD, 28.3% had no effect and 16.3% shortened APD. Of the 70 compounds without effect on hERG channels, 54.3% did not affect APD, 25.7% prolonged, while 20% significantly shortened APD. Dofetilide (hERG inhibitor; IC₅₀, 29 nM) prolonged QT and elicited early after-depolarizations and/or torsade de pointes (TdP) in isolated hearts. Mallotoxin and NS1643 (hERG current stimulators at 3 μM), levromakalim and nicorandil (no effect on hERG current), all significantly shortened APD and QT, and elicited ventricular fibrillation (VF) in isolated hearts.

Conclusion and implications: The hERG assay alone did not adequately identify drugs inducing QT prolongation. It is also important to detect drug-induced QT shortening, as this effect is associated with a potential risk for ventricular tachycardia and VF, the latter being invariably fatal, whereas TdP has an ~15–25% incidence of death.

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Keywords: action potential duration; hERG; human ether-a-go-go-related gene; K⁺ channel; QT prolongation; short QT; VF; ventricular fibrillation

Abbreviations: APD, duration of the action potential; hERG, human ether-a-go-go-related gene; ICH, International Conference on Harmonisation; I_{Kr} , the rapidly activating delayed rectifier potassium current; K_{ATP}⁺, ATP-sensitive potassium current; Tp-Te, transmural dispersion; TdP, torsade de pointes; VT, ventricular tachycardia; VF, ventricular fibrillation

Introduction

In the heart, drug-induced prolongation of the QT interval and the appearance of torsade de pointes (TdPs) are recognized as potential risks associated with the use of a broad range of cardiovascular and non-cardiovascular drugs (Haverkamp *et al.*, 2000; Cubeddu, 2003; Shah, 2004). Current established regulatory guidelines (CPMP/986/96,

1997 and International Conference on Harmonisation (ICH) S7B, 2005) recommend the use of preclinical studies to detect drug-induced QT interval prolongation and arrhythmogenic potential.

The most common mechanism for drugs that induce prolongation of the QT interval is the inhibition of the rapidly activating delayed rectifier potassium current (I_{Kr}) of the cardiac cells. I_{Kr} is the key current responsible for phase 3 repolarization of the cardiac action potential and is conveyed by the human ether-a-go-go-related gene-encoded, voltage-dependent potassium channel (hERG channel). As such, the current regulatory guidelines recommend the hERG test for identifying drugs with potential QT liabilities.

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Drug-induced long QT and its risk for TdPs are now well known because, in recent years, extensive progress has been made in studying the congenital long QT syndromes (Roden, 2008), and also in drug-induced TdP through different screening strategies, evaluated following the release of CPMP/986/96 in 1997. Conversely, little is known about genetic short QT syndromes, characterized by abnormally short QT intervals (<300 ms), atrial fibrillation, palpitations, syncope and sudden death due to ventricular fibrillation (VF) (Gaita *et al.*, 2003; Giustetto *et al.*, 2006). As it is difficult to capture QT shortening in the setting of the associated fatal arrhythmia (VF), knowledge concerning drug-induced short QT intervals is sparse, with only a few publications, suggesting that a short QT interval could potentially increase the risk of sudden death (D'Alonzo *et al.*, 1994, 1998; Extramiana and Antzelevitch, 2004; Hondeghem, 2006; Garberoglio *et al.*, 2007). Indeed, the present ICH S7B guideline (Food & Drug Administration, 2005) does not specifically address the possibility of a drug-induced shortening of the QT interval and its potential for development of fatal arrhythmias.

Our present study was carried out on over 500 bioactive compounds from Johnson & Johnson's current research programmes, and also included 5 reference compounds—dofetilide, mallotoxin, NS1643, levcromakalim and nicorandil—with known mechanisms of action. Our findings showed that an appreciable number of compounds did not affect the hERG channel, but did prolong the action potential duration (APD). Additionally, although many compounds did prolong APD and QT, a relatively large number of compounds shortened APD and QT, and several of these could precipitate ventricular tachycardia (VT) and VF. These observations identify a serious gap in the design of the present guidelines to identify and eliminate potentially arrhythmogenic compounds. Some of the data in this paper have been published in abstract form (Lu *et al.*, 2008).

Materials and methods

All experimental procedures were performed in accordance with the provisions of the European Convention on the protection of vertebrate animals, which are used for experimental and other scientific purposes, and with 'the Appendices A and B', made at Strasbourg on March 18, 1986 (Belgian Act of October 18, 1991).

Effects of drugs on hERG current

The experimental approach we used is similar to that employed in other studies (Volberg *et al.*, 2002; Dubin *et al.*, 2005; Lu *et al.*, 2007). A human embryonic kidney cell line (HEK293) with a stable transfection of hERG was used (purchased from Dr Z Zhou, University of Wisconsin, Madison, WI, USA). The cells were cultured in MEM (Minimum Essential Medium, GIBCO, Invitrogen Corporation; Carlsbad, USA) supplemented with (amounts added to 500 ml MEM): 5 ml L-glutamine–penicillin–streptomycin (Sigma, St Louis, MO, USA), 50 ml foetal bovine serum (Bio-Whittaker, Walkersville, USA), 5 ml non-essential amino

acids 100 × (GIBCO), 5 ml sodium pyruvate 100 mM (GIBCO) and 4 ml geneticin 50 mg ml⁻¹ (GIBCO) at 37 °C in 5% CO₂ atmosphere. Before use, the cells were seeded in small Petri dishes precoated with poly-L-lysine (Biocoat, Becton Dickinson Labware; Bedford, USA). Experiments were carried out 1–2 days after plating.

Conventional patch-clamp experiments. Recordings were conducted at room temperature. Solutions used were as follows: the bath solution contained (in mM) 150 NaCl, 4 KCl, 1 MgCl₂, 1.8 CaCl₂, 10 HEPES, 5 glucose (pH 7.4 with NaOH). The pipette solution contained (in mM) 120 KCl, 10 HEPES, 5 EGTA, 4 ATP-Mg₂, 2 MgCl₂, 0.5 CaCl₂ (pH 7.2 with KOH). Solutions were applied to the cell under study using a Y-tube system, allowing a rapid delivery and mixing of solutions in the vicinity of the cell under study. The membrane current of the cells was measured at distinct membrane potentials by means of an EPC-9 patch clamp amplifier (HEKA, Lambrecht/Pfalz, Germany). Data were acquired and analysed using the programs Pulse and PatchmasterPro (HEKA, HEKA Elektronik, GmbH; Lambrecht/Pfalz, Germany), Data Access (Bruyton, Seattle, USA), Igor (Wavemetrics, Lake Oswego, USA) and Excel (Microsoft, Redmond, USA). After disruption of the membrane, the cell capacitance and the series resistance were compensated (90%) using the circuit of the EPC-9 patch clamp amplifier. The holding potential was -80 mV. The hERG-mediated current (K⁺-selective outward current) was determined as the maximal tail current at -40 mV after a 2 s depolarization to +60 mV. Pulse cycling rate was 15 s. Before each test pulse, a short pulse (0.5 s) to -60 mV was given to determine leak current. The protocol consisted of a 5 min equilibration period (no pulses), 5 min in control solution and then 5 min for each concentration of the drug. Up to three concentrations were tested per cell (0.001–100 μM). Rundown of the hERG current was taken into account by extrapolating the gradual decrease in current measured during the 5 min in control solution to the remaining duration of the experiment. The effect of the drug was measured after 5 min of drug application by dividing the measured current by the extrapolated current.

In addition to the conventional patch clamp, for some compounds, the hERG test was performed by an automated patch clamp system (PatchXpress 7000A). This experimental approach was similar to that described earlier (Dubin *et al.*, 2005).

Effects of drugs in isolated rabbit Purkinje fibres

Electrophysiological experiments were performed on isolated Purkinje fibres from female rabbits, using conventional microelectrode techniques. The approach is similar to that used in recent studies from our laboratories (Lu *et al.*, 2001). Briefly, Purkinje fibres were isolated from left ventricles within 5 min after the death of the animal and stored in a tissue bath. The cardiac tissues were perfused with gassed (95% O₂ and 5% CO₂) Tyrode's solution of the following composition (in mM): NaCl 136.9, KCl 4, CaCl₂ 1.8, MgCl₂ 1.04, NaHCO₃ 11.9, NaH₂PO₄ 0.42 and glucose 5.5. The preparations were fixed at the bottom of a 2.5-ml perfusion

organ bath by two small needles and continuously superfused with oxygenated Tyrode's solution at a rate of 2.7 ml min^{-1} together with solutions of the reference compounds or solvent at a rate of $300 \mu\text{l min}^{-1}$. The temperature in the bath was kept at $35.5 \pm 1.0^\circ\text{C}$.

The Purkinje fibres were stimulated at a basal rate of 1 Hz (60 beats per minute or 1000 ms cycle length) through bipolar Teflon-coated silver wire electrodes that were connected to a pulse generator and an isolation transformer. Stimuli consisted of regular pulses of 0.5–1 ms duration, delivered at an intensity of two times the diastolic threshold. Intracellular potentials were recorded with glass microelectrodes filled with 2.7 M KCl, with tip resistances between 5 and 65 M Ω . The microelectrode was connected to the headstage of the microelectrode amplifier (Hugo Sachs Elektronik: HSE; type 695; March-Hugstetten, Germany). Action potential signals were acquired by action potential software (Notocord-Hem 3.5, Paris) at a sampling rate of 20 kHz, filtered at 100 Hz. The signals were stored on a personal computer for subsequent analysis. The optimal stimulation site of the preparation was determined as the site where a normal and stable action potential could be induced. The maximum rate of rise of the action potential was obtained by electronic differentiation (V_{max} in V s^{-1}). The amplitude of action potential (AAP in mV), and APD at 40, 50 or 90% repolarization (APD₄₀, APD₅₀ or APD₉₀ in ms) were determined from the recordings.

Control values (baseline values before contact with solvent or compound) were determined after a stabilization period, when the measured variables had reached a steady state. The electrophysiological parameters were again measured during the experimental period.

After baseline values had been recorded at 1 Hz, solvent or one of the testing compounds at four concentrations (0.01–10 μM or 0.1–100 μM) ($n=5-7$ for each compound) was continuously superfused for 15 min for each concentration at a stimulation rate of 1 Hz. In addition, the stimulation rate was reduced to 0.2 Hz at the highest concentration for another 5 min, because preparations are more prone to induction of early after depolarizations in a condition of extreme bradycardia (Lu *et al.*, 2001).

Effects of drugs in isolated Langendorff-perfused rabbit hearts

The method for determining various cardiac electrophysiological properties was similar to, and has been described in detail as, the isolated, Langendorff-perfused rabbit heart (Hondeghem *et al.*, 2003; Lu *et al.*, 2007). Briefly, Langendorff experiments were performed on the hearts isolated from about 2.5 kg female albino rabbits. The heart was perfused at a constant pressure of 80 cm H₂O with a bicarbonate buffer (in mM: NaCl 118, KCl 3.5, NaHCO₃ 22, MgCl₂ 1.1, NaH₂PO₄ 0.4, CaCl₂ 1.8, glucose 5, pyruvate 2 and creatine 0.038, gassed with 95% O₂ and 5% CO₂, pH adjusted to 7.4, at 37 °C). Under a dissection microscope, the bundle of His was cut and a stimulating electrode sutured on each side of the distal His bundle. A recording electrode was advanced until it reached the left ventricular endocardium of the septum.

Stimulation current was adjusted, and automaticity, escape cycle lengths, conduction times and APDs for cycle lengths

at 2000, 1500, 1000, 750, 500, 300 and 250 ms were determined. Trains of 5 s at cycle lengths of 250, 500, 750 and 1000 ms were recorded. APD₉₀ or APD₆₀, triangulation (APD₉₀–APD₃₀), short-term beat-to-beat instability of the APD₉₀ (Lu *et al.*, 2007), conduction time and coronary flow were measured during experimental periods. A bath ECG from the Lead II, reflecting the propagation of action potential waves Q, R, S, J and T, was placed and recorded. ECG parameters such as QRS duration, QT interval, JT interval, transmural dispersion (Tp–Te) [= QT_{peak}–QT_{tend}], rTp–Te (Tp–Te/QT-interval \times 100) (Shimizu and Antzelevitch, 1997; Yan *et al.*, 2001; Extramiana and Antzelevitch, 2004) were also measured. Conduction time was measured as the interval between the electrical stimulation to the point where the action potential just started the depolarization (upstroke of the action potential).

The solvent or drug solutions (four concentrations of the testing compound from 0.001 to 100 μM ; $n=6-8$ for each compound) were perfused for 30 min per concentration.

Monophasic action potentials from the inside of the left ventricle, the right and left epicardium and ECG were recorded and digitized at 1 kHz (12 bits). For the conduction data, sampling was done at 10 kHz (each channel). Data were analysed beat by beat during the experiment, and the results were compressed and saved to disc.

Data analysis

All values are expressed as mean and s.e.mean. Statistically significant differences between solvent and compound were calculated based on their changes from baseline with the Mann–Whitney *U*-test. For the evaluation of differences in the incidence of VF, Fisher's exact test was used. Two-tailed probabilities of less than 0.05 were considered to indicate statistically significant differences.

Materials

A total of 635 novel compounds from Johnson & Johnson's worldwide research projects were used. Reference compounds such as levcromakalim, nicorandil and mallo-toxin were obtained from Sigma (St Louis, MO, USA), and NS1643 was gratefully received from Dr M Grunnet (Neuro-Search A/S, Ballerup, Denmark). The compounds were dissolved in pyrogen-free water (acidified with tartaric acid to obtain a pH of approximately 4), buffer solution or in dimethyl sulphoxide (final bath concentration of 0.1 or 0.3%). Similar solutions without compound were used as solvent controls.

Results

Criteria for the assessment of effects of compounds on hERG current and on APD in vitro

The effect of each compound, on the variables measured, was expressed as the solvent-corrected net effect. An effect was defined as 'hERG inhibition' if a compound inhibited hERG current by $\geq 20\%$ at a concentration $\leq 3 \mu\text{M}$, as 'hERG stimulation' (or activation of hERG) if a compound

activated hERG current $\geq 10\%$ at a concentration $\leq 3 \mu\text{M}$ or was considered as 'no effect' if a compound was associated with $< 20\%$ inhibition or $< 10\%$ activation of hERG current at a concentration $\leq 3 \mu\text{M}$ ($n=3-5$ per compound and per concentration) or effects appeared only at high concentrations ($> 3 \mu\text{M}$). An effect was defined as a prolongation of the APD if the compound induced $\geq 10\%$ prolongation of APD at 90% repolarization (APD₉₀) in isolated Purkinje fibres or of APD at 60% repolarization (APD₆₀) in isolated, Langendorff-perfused hearts, at any tested concentration ($n=6-8$). An effect was defined as a shortening of APD if the compound induced $\geq 10\%$ shortening of APD₉₀ or APD₆₀ at any tested concentrations. The compound was considered as having no effect if it induced a change in APD₉₀ or APD₆₀ between -10% and $+10\%$ change from the baseline value.

Effects on hERG

A total of 576 compounds were tested in the hERG test. Of these, 58% ($n=333$) inhibited hERG current, 39% ($n=226$) had no effect on the hERG current and 3% ($n=17$) were found to stimulate hERG current (upper part of Figure 1).

Effects on APD

A total of 229 compounds were screened in the action potential tests (isolated Purkinje fibres or isolated Langendorff-perfused hearts). Of these, 44% ($n=100$) were found to prolong the duration of the action potential, 39% of these 229 compounds ($n=89$) had no effect on APD, whereas 17% ($n=40$) were found to significantly shorten APD (lower part of Figure 1).

Effects on hERG and APD

A total of 170 compounds were tested in both the hERG assay and an action potential assay (isolated Purkinje fibres or isolated Langendorff-perfused hearts). Of these 170 compounds, 54% ($n=92$) were found to block hERG current, 41% ($n=70$) were without effect and 5% ($n=8$) were found to activate hERG current.

Of the 92 compounds inhibiting hERG current, 55.4% ($n=51$) prolonged APD, 16.3% ($n=15$) shortened APD and 28.3% ($n=26$) did not change APD (upper part of Figure 2). Of the 70 compounds without effect in the hERG test, 54.3% ($n=38$) did not change APD, 20% ($n=14$) shortened APD, but surprisingly 25.7% ($n=18$) still prolonged APD (middle part of Figure 2).

Of the eight compounds found to be hERG stimulators or 'activators', three had no effect on APD, two prolonged APD, whereas three compounds, including NS1643 and mallotoxin, shortened APD (lower part of Figure 2).

Effects of reference compounds on hERG

Levcromakalim had no effect on hERG current up to a concentration of $10 \mu\text{M}$, and nicorandil did not affect hERG current up to a concentration of $100 \mu\text{M}$. As expected, mallotoxin activated hERG current by 87% at $3 \mu\text{M}$, and

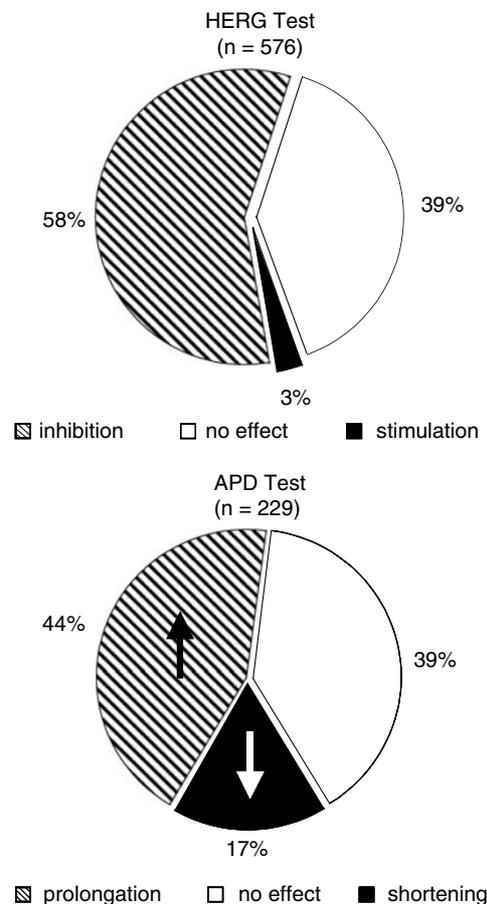


Figure 1 Upper part: effects of 576 compounds on the hERG current. Inhibition: $\geq 20\%$ inhibition of hERG current at a concentration $\leq 3 \mu\text{M}$; no effect: $< 20\%$ inhibition or $< 10\%$ activation of hERG current at a concentration $\leq 3 \mu\text{M}$ or effects appeared only at high concentrations ($> 3 \mu\text{M}$); hERG stimulation (activation): $\geq 10\%$ activation of hERG at a concentration $\leq 3 \mu\text{M}$ ($n=3-5$ per concentration). Lower part: effects of 229 compounds on the duration of the action potential at 60 or 90% repolarization (APD₆₀ or APD₉₀) on isolated rabbit Purkinje fibres or on isolated Langendorff-perfused rabbit hearts. 'Prolongation' was defined as a prolongation of the action potential duration (APD) if the compound induced $\geq 10\%$ prolongation of APD at 90% repolarization (APD₉₀) in isolated rabbit Purkinje fibres or of APD at 60% repolarization (APD₆₀) in isolated, Langendorff-perfused rabbit hearts, at any tested concentration ($n=6-8$). 'Shortening' was defined as a shortening of APD if the compound induced $\geq 10\%$ shortening of APD₉₀ or APD₆₀ at any tested concentrations. 'No effect' was defined as an effect between -10% and $+10\%$ change from baseline in APD₉₀ or APD₆₀.

NS1643 activated hERG current by 55% at $3 \mu\text{M}$. Dofetilide blocked hERG current with an IC₅₀ of 29 nM. Figure 3 shows an example of the effects of dofetilide and NS1643 in the hERG assay.

Effects of five reference compounds on the action potential: in isolated Purkinje fibres or in isolated Langendorff-perfused hearts
Dofetilide ($n=7$), which inhibited hERG current with an IC₅₀ of 29 nM, concentration-dependently prolonged APD in isolated Purkinje fibres, increased triangulation of the action potential and elicited early after-depolarizations starting at a concentration of $0.01 \mu\text{M}$. (example on the right side of

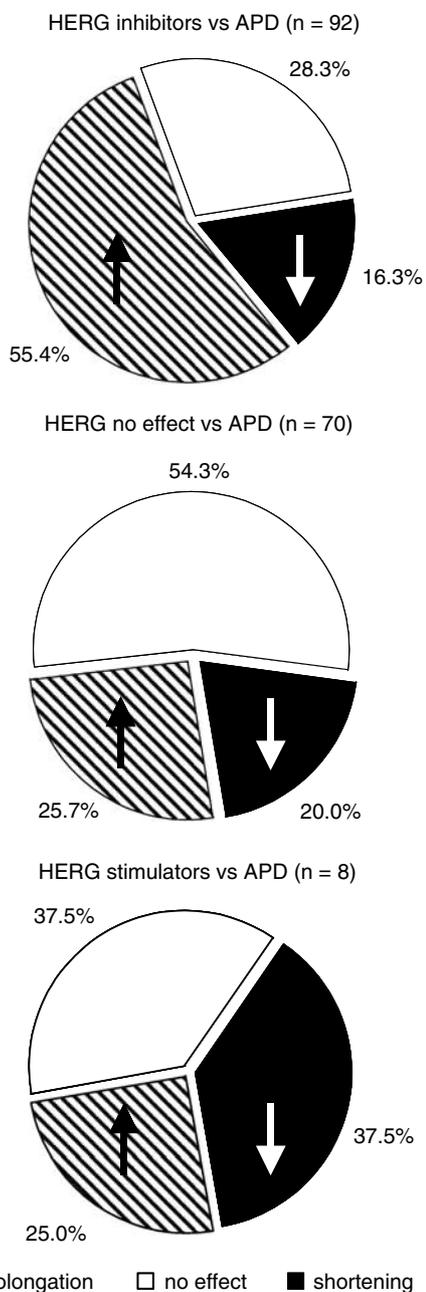


Figure 2 Effects of a selection of compounds with known effects in the hERG test, on APD (isolated Purkinje fibres or isolated Langendorff-perfused hearts). Upper part: hERG inhibitors. Middle part: compounds without effect on hERG. Lower part: hERG stimulators.

Figure 4). In the isolated Langendorff-perfused heart, dofetilide ($n = 7$) significantly prolonged the APD₉₀ starting at 0.001 μM and QT interval starting at 0.01 μM , and tended to increase Tp-Te starting at a concentration of 0.01 μM . The compound significantly increased the triangulation of the action potential at 0.01 μM and the beat-to-beat instability at 0.1 μM and elicited early after-depolarizations and TdP starting at 0.01 μM (example on the left side of Figure 4).

The other reference compounds (maltotxin, NS1643, levromakalim and nicorandil) are all known to shorten

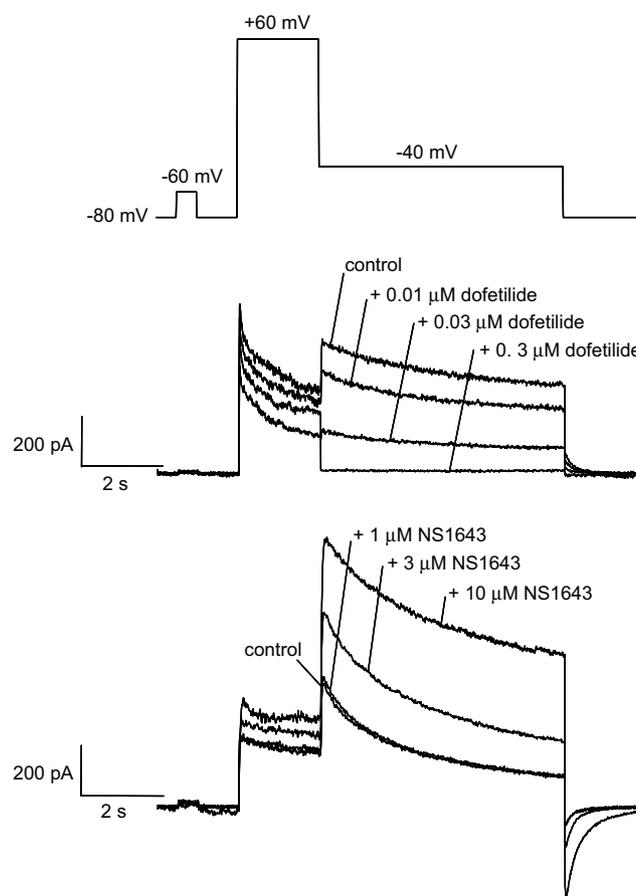


Figure 3 Effect of dofetilide and NS1643 on the membrane K⁺ current (I_{Kr}) in hERG-transfected HEK293 cells. Upper part: schematic representation of the pulse protocol: a small test pulse to -60 mV to determine the leak current, a depolarizing pulse to $+60$ mV to activate the K⁺ current and elicit the large tail current (which is used to determine the amplitude of the K⁺ current I_{Kr}). Finally, the cells are clamped back to the holding potential of -80 mV. This protocol is repeated every 15 s. Lower part: superimposed current traces in one cell in different conditions: solvent control solution and increasing concentrations of dofetilide or NS1643. Dofetilide inhibited hERG current with an IC₅₀ of 29 nM and NS1643 activated hERG current by 55% at 3 μM .

APD and QT interval and were only studied in the isolated heart. Maltotxin, which activated hERG current by 87% at 3 μM in hERG-transfected HEK293 cells, significantly shortened the QT interval, JT interval and APD and increased Tp-Te and rTp-Te and coronary flow at 0.1 μM . At 1 μM , maltotxin elicited VT in 2 out of the 7 hearts and VF in 7 out of the 7 hearts (versus 0 out of the 12 hearts with solvent). These effects are illustrated by the experimental results shown in Figure 5.

NS1643, which activated hERG current by 55% at 3 μM in hERG-transfected HEK293 cells, significantly shortened APD and increased coronary flow at 1 and 10 μM . At 10 μM , the compound shortened the QT and JT interval and tended to increase the Tp-Te. Furthermore, NS1643 elicited VT in one out of the six hearts at 1 μM and VF in four out of the six hearts at 10 μM .

Levromakalim, which did not affect hERG current at the concentrations tested, was also studied in detail in the

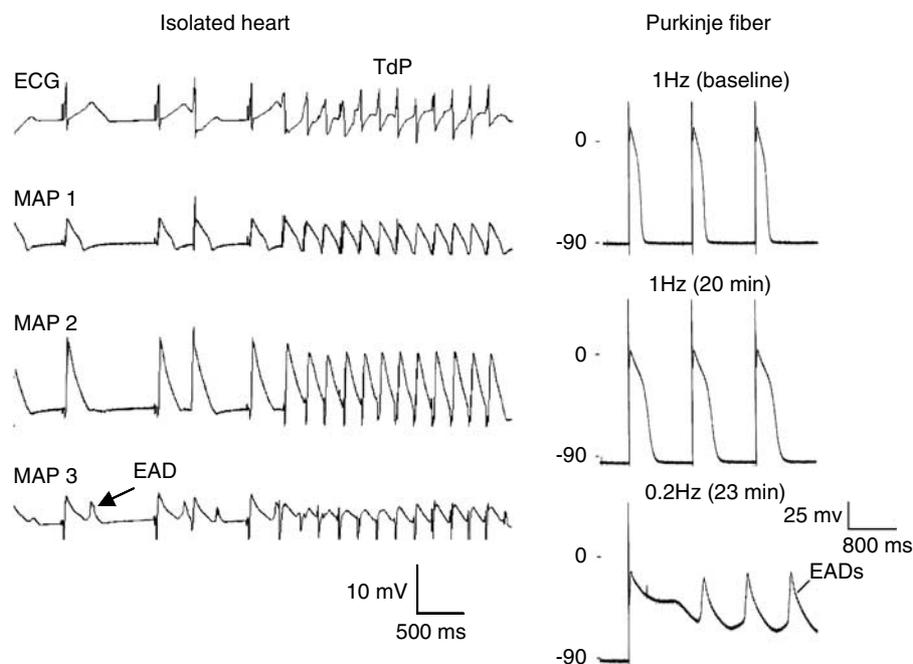


Figure 4 The effect of dofetilide on action potential morphology in an isolated Purkinje fibre (right side of the figure) and on ECG and MAP recordings in the isolated heart. The compound prolonged the duration of the action potential (APD) and elicited early after-depolarizations (EADs) at a stimulation rate of 0.2 Hz at 0.01 μM in the Purkinje fibre, and prolonged the QT interval/APD and elicited EAD and TdP at 0.01 μM in an isolated Langendorff-perfused heart. ECG: electrocardiogram recording; MAP1, the left ventricular epicardial monophasic action potential (MAP) recording; MAP2, right ventricular MAP recording; MAP3, left intra-ventricular MAP recording.

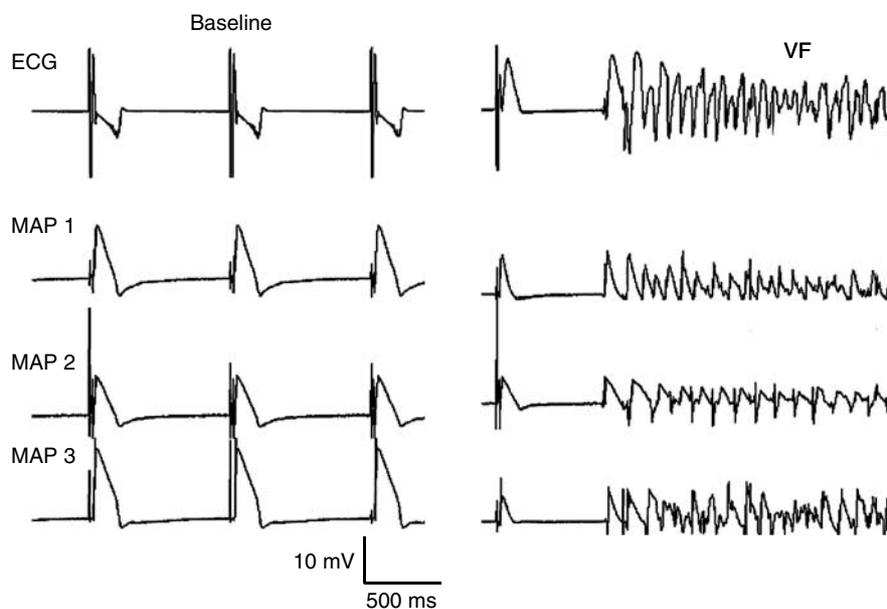


Figure 5 The effect of mallotoxin on ECG and MAP recordings in the isolated heart. At 1 μM , mallotoxin shortened QT and the duration of the action potential and elicited ventricular fibrillation (VF). ECG, electrocardiogram recording; MAP1, the left ventricular epicardial monophasic action potential (MAP) recording; MAP2, right ventricular MAP recording; MAP3, left intra-ventricular MAP recording. T wave was inverted and narrowly peaked.

isolated Langendorff-perfused heart. Levromakalim shortened QT interval and APD and increased coronary flow at 0.1 μM and increased rTp-Te at 1.0 μM . At 10 μM , levromakalim markedly changed these parameters. Furthermore, the compound elicited VT in three out of eight hearts at 1 μM , and VF in seven out of eight hearts at 10 μM . Figure 6 shows

the shortening of the QT and JT intervals and of APD and the induction of VT in an isolated Langendorff-perfused heart in the presence of 1 μM levromakalim.

Nicorandil, like levromakalim, did not affect hERG current. However, nicorandil slightly shortened the QT and JT intervals and APD, increased coronary flow, Tp-Te and

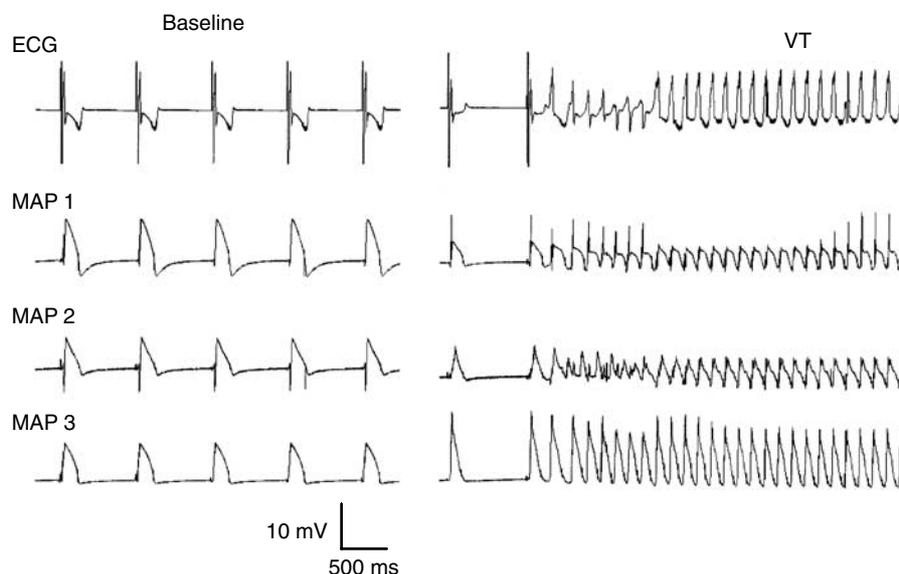


Figure 6 The effect of levromakalim on ECG and MAP recordings in the isolated heart. Levromakalim shortened APD and elicited ventricular tachycardia (VT) at 1 μ M. ECG, electrocardiogram recording; MAP1, the left ventricular epicardial monophasic action potential (MAP) recording; MAP2, right ventricular MAP recording; MAP3, left intra-ventricular MAP recording.

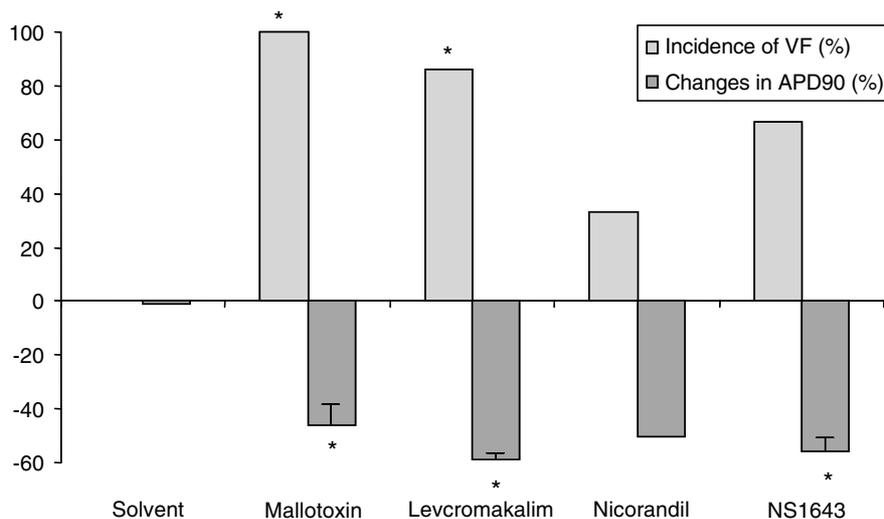


Figure 7 Drug-induced effects on APD₉₀ (values were taken just before the induction of VF and expressed as % changes from baseline) and incidence of ventricular fibrillation (VF) (in %) in isolated, Langendorff-perfused hearts. Data of mallotoxin ($n=7$) were obtained at 1 μ M, NS1643 ($n=4$) and levromakalim ($n=7$) were obtained at 10 μ M and nicorandil ($n=2$) was obtained at 100 μ M. APD₉₀: the duration of the action potential at 90% repolarization. Values were taken from the hearts with VF. * $P < 0.05$ versus solvent.

rTp-Te, and elicited VT in one out of six hearts and VF in only two out of the six hearts at a higher concentration of 100 μ M.

The effects of the four compounds on the incidence of VF, APD, the duration of the QT and JT intervals, Tp-Te, rTp-Te and beat-to-beat instability before VF are summarized in Figures 7–10. The incidence of VF by the compounds was associated with shortening in the ventricular repolarization (QT-interval, JT-interval and APD₉₀) and increases in the transmural dispersion (Tp-Te and rTp-Te), just before the VF. These four compounds, however, did not significantly change ventricular conduction time or triangulation of the action potential (data not shown).

Discussion and conclusions

The hERG (I_{Kr})-testing paradigms recommended in the present regulatory guidelines (ICH S7B, 2005) for predicting drug-induced QT prolongation may not adequately describe all the conditions needed to efficiently eliminate those compounds able to induce QT prolongation and hence TdP. Even more concerning is the fact that the ICH guidelines fail to address the possibility of precipitation of other, potentially more life threatening, drug-induced cardiac arrhythmias such as VT and VF, associated with drug-induced shortening of the QT interval.

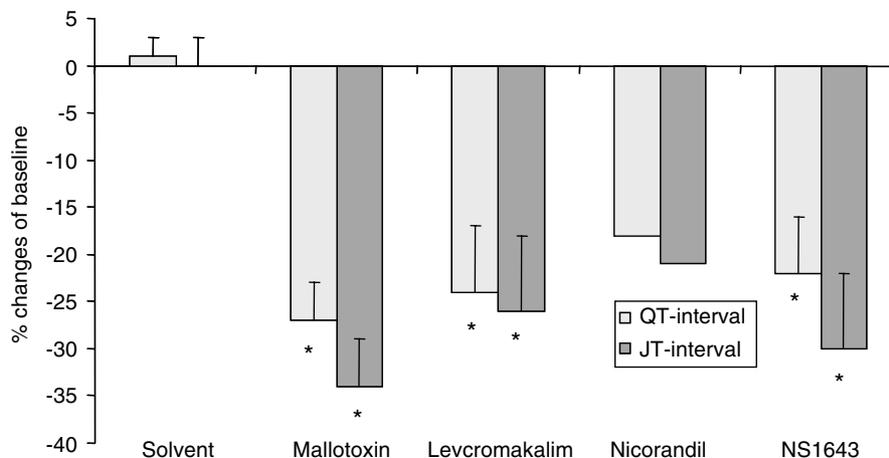


Figure 8 Effects of mallotoxin ($1 \mu\text{M}$; $n=7$), levromakalim ($10 \mu\text{M}$; $n=7$), nicorandil ($100 \mu\text{M}$; $n=2$) and NS1643 ($10 \mu\text{M}$; $n=4$) on the durations of the QT and JT intervals in isolated, Langendorff-perfused hearts, just before the occurrence of ventricular fibrillation (VF). Values were taken from the hearts with VF and expressed as % changes from baseline. * $P < 0.05$ versus solvent.

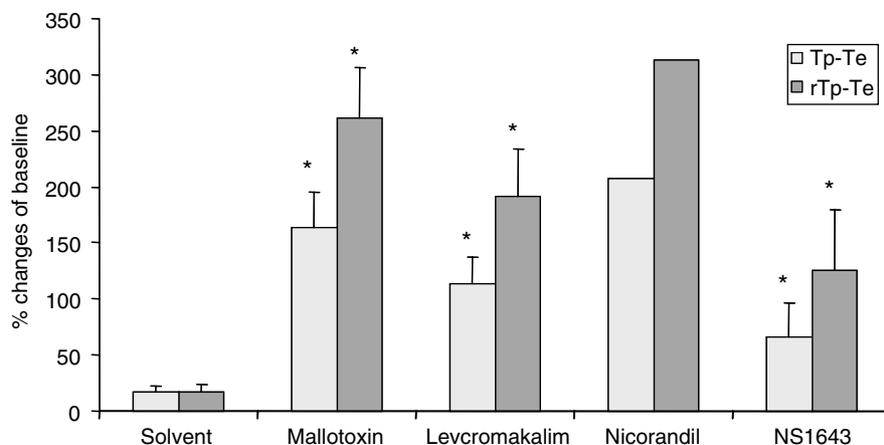


Figure 9 Effects of mallotoxin ($1 \mu\text{M}$; $n=7$), levromakalim ($10 \mu\text{M}$; $n=7$), nicorandil ($100 \mu\text{M}$; $n=2$) and NS1643 ($10 \mu\text{M}$; $n=4$) on Tp-Te and rTp-Te (transmural dispersion of the ventricular repolarization) in isolated, Langendorff-perfused hearts just before the ventricular fibrillation (VF). Values were taken from the hearts with VF and expressed as % changes from baseline. * $P < 0.05$ versus solvent.

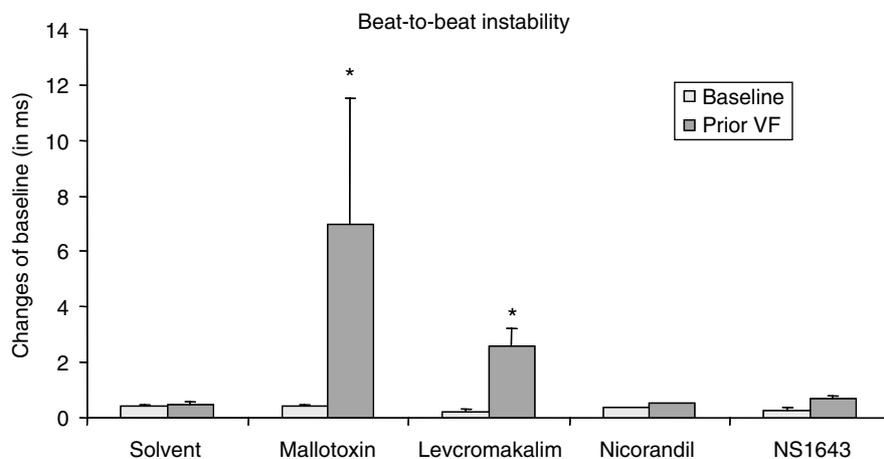


Figure 10 Effects of mallotoxin ($1 \mu\text{M}$; $n=7$), levromakalim ($10 \mu\text{M}$; $n=7$), nicorandil ($100 \mu\text{M}$; $n=2$) and NS1643 ($10 \mu\text{M}$; $n=4$) on the beat-to-beat instability of the action potential in isolated, Langendorff-perfused hearts just before the ventricular fibrillation (VF). Values of solvent were taken at the baseline and the last time-matched point without VF. * $P < 0.05$ versus baseline or solvent.

Clearly there is a consensus about the importance of drug interactions with the hERG ion channel and numerous papers confirm that inhibition of hERG (I_{Kr}) is a common feature of many drugs capable of inducing TdP in man (Haverkamp *et al.*, 2000; Cubeddu, 2003; Shah, 2004). Therefore, the blockade of hERG current remains the predominant risk factor in drug-induced TdP. Figure 3 shows that dofetilide inhibited hERG current with an IC_{50} of 29 nm, markedly prolonged APD in isolated Purkinje fibres and largely prolonged APD and QT and elicited TdP in isolated hearts (Figure 4). In the clinic, the concept of drug-induced TdP, resulting from hERG channel inhibition, is supported by the congenital long QT syndrome (LQT2) which is manifested by mutations of the hERG gene (Chiang and Roden, 2000). However, despite possessing such a mutation, not all patients with a variant hERG channel present with a clear long QT interval. Furthermore, mutations in other genes for cardiac ion channels such as I_{Ks} (the slowly activating delayed rectifier potassium current) or SCN5A (encodes for the cardiac voltage-gated Na^+ channel) can also lead to long QT in man, which sometimes results in TdPs. Thus, unravelling the mechanisms underlying congenital long QT syndromes suggests that factors other than hERG channel inhibition and QT prolongation may also be involved in drug-induced TdP. Therefore, although the hERG test to predict drug-induced long QT is very useful, this test alone may not be able to ascertain fully the potential for precipitation of TdP.

In our present study we have shown that (1) not all drugs that block the hERG ion channel prolong QT and APD, and that (2) some drugs that do not inhibit the hERG channel are not necessarily free of the risk of drug-induced long QT and TdP (Figure 2). Compounds that potently block the hERG current, but normally do not prolong QT and APD, include Ca^{2+} blockers (such as verapamil) and some Na^+ channel blockers. Thus, flecainide inhibits hERG currents by 81% at 10 μ m and lidocaine inhibits hERG by 25% at 100 μ m. Indeed, in this study, 28.3% of the hERG inhibitors tested in an APD assay did not prolong repolarization. On the other hand, compounds that prolong QT and APD, but have no effect on the hERG current *in vitro* include those that prolong repolarization via another mechanism (see Lu *et al.*, 2007; Lacerda *et al.*, 2008): (1) inhibition of I_{Ks} current (such as with HMR 1556); (2) inhibition of I_{to} current (transient outward current) (such as with 4-aminopyridine); (3) blockade of I_{K1} current (such as with $BaCl_2$); (4) an increase in inward depolarizing current (Ca^{2+} current, such as with Bay K 8644); (5) augmentation of late I_{Na} current (sodium current) through application of veratridine or ATX-II (Shimizu and Antzelevitch, 1997). Based on data from the Cardiovascular Safety Department within Johnson & Johnson, 25.7% of the compounds that did not affect hERG-mediated current increased the APD. In these cases, the standard hERG test alone is not able to reliably provide a mechanism for APD and QT prolongation *in vitro* or *in vivo*. In addition, such studies may be limited by the physicochemical properties of the compound (that is, poor solubility) for use in such *in vitro* experiments or may be absorbed by the glass and plastic surfaces used in the experimental apparatus, resulting in exposure of the cells to artefactually lower concentrations.

Drug-induced short QT and possible mechanisms

Since 2000, there has been increasing evidence that short QT syndromes constitute a new primary electrical abnormality with high incidence of sudden death, resulting not from TdPs, but rather from VF (Gussak *et al.*, 2000; Gaita *et al.*, 2003; Schimpf *et al.*, 2005). However, the mechanism(s) responsible for drug-induced or acquired short QT syndromes are much less known.

The short QT syndrome is characterized by familial sudden death, short ventricular refractory periods and inducible VF, even in young patients and newborns (Borggreffe *et al.*, 2005; Giustetto *et al.*, 2006). 'Gain-of-function' mutations in three different genes encoding cardiac potassium channels (specifically $KCNH2$, $KCNQ1$ and $KCNJ2$, which are responsible for an increase in I_{Kr} current) (Borggreffe *et al.*, 2005) as well as 'loss-of-function' mutations in genes encoding the cardiac L-type calcium channel (Antzelevitch *et al.*, 2007) have been associated to date with the congenital short QT syndrome. In all these cases, there is a resulting increase in ventricular repolarizing current leading to a shortening of the APD, subsequent reduction in the ventricular refractory period and, as a consequence, a shortening of the QT interval. Although the congenital short QT has only been recognized recently, the existence of acquired short QT has been known for a long time. It has been shown that acquired short QT intervals can result from altered electrolyte levels such as hypercalcemia, hyperkalemia and myocardial ischaemia and may also result from elevated acetylcholine and catecholamine plasma levels, hyperthermia and tachycardia (Nierenberg and Ransil, 1979; Karjalainen and Viitasalo, 1986). However, although such physiological mechanisms have been examined, the mechanisms responsible for a drug-induced short QT interval have not been adequately elucidated or defined. In this study, we confirm at least two mechanisms through which drugs can shorten QT and APD and induce VT and VF, that is, activation of the ATP-sensitive potassium current (K_{ATP}^+) channel and activation of hERG current (I_{Kr}). Drug-induced short QT both by K_{ATP}^+ channel openers and I_{Kr} activators, on the other hand, can be anti-arrhythmic under certain conditions, both in man (Shimizu *et al.*, 1998) and in an experimental setting (Hansen *et al.*, 2007).

K_{ATP}^+ channel openers are already known to accelerate ventricular repolarization resulting from a shortened APD and effective refractory period (Escande *et al.*, 1989; Robert *et al.*, 1997; Milberg *et al.*, 2007). Furthermore, the activation of the K_{ATP}^+ channel is potentially pro-arrhythmic as it elicits VT and VF *in vitro* in isolated hearts (Robert *et al.*, 1997, 1999; Milberg *et al.*, 2007) and *in vivo* (de La Coussaye *et al.*, 1993; Wilde, 1994). Indeed, the activation of the K_{ATP}^+ channel with pinacidil shortened the QT interval, increased maximal transmural dispersion of repolarization or dispersion of the repolarization between the left and right ventricles and elicited VT with a programmed extra stimulus (S2) in the dog ventricular wedge preparation and isolated rabbit heart (Extramiana and Antzelevitch, 2004; Milberg *et al.*, 2007). In this study, we investigated potential arrhythmogenic effects, in isolated rabbit hearts, of two K_{ATP}^+ channel openers (levcromakalim and nicorandil; Escande *et al.*, 1989; Robert *et al.*, 1997 and Milberg *et al.*, 2007). These two compounds,

which have no effect on hERG current, shortened the QT interval and APD, and increased Tp-Te and rTp-Te (parameters of transmural dispersion) (Shimizu and Antzelevitch, 1997; Yan *et al.*, 2001), instability of the action potential (Figures 8–10) and coronary flow (the latter through their coronary vasodilator action). The effects were most pronounced with levromakalim and were associated with an incidence of VF of 87.5% in the isolated hearts. The low incidence of arrhythmia with nicorandil was only observed at a very high concentration (100 μM), which is likely to be much higher than the therapeutic plasma levels of the compound achievable in man.

The inhibition of the hERG cardiac K^+ channel leads to action potential prolongation on the cellular level, a prolongation of the QT interval on the electrocardiogram and, occasionally, cardiac arrhythmias, whereas the activation of hERG current (I_{Kr}) with mallotoxin or NS1643 was recently shown to shorten APD (Zakharov *et al.*, 2005; Casis *et al.*, 2006; Hansen *et al.*, 2006; Zeng *et al.*, 2006). Furthermore, increasing I_{Kr} by overexpression of hERG currents, either by adenoviral transfer in the rabbit or guinea pig or by transgenic modification in mouse was reported to shorten APD (Nuss *et al.*, 1999; Royer *et al.*, 2005). However, the relationship between drug-induced shortening of APD and QT interval by the activation of I_{Kr} current and proarrhythmic events such as VT/VF is not well understood. In this study, we showed that both mallotoxin and NS1643 activated hERG current by 87% and 55% at 3 μM , respectively.

Mallotoxin is a relatively non-specific cardiac ion channel activator. In addition to hERG channel activation, the toxin (at 0.5 μM) also activates Ca^{2+} -activated K^+ channels in HEK cells stably expressing the BK channel (SLO1) (Zakharov *et al.*, 2005). Furthermore, mallotoxin is also a protein kinase C and calcium/calmodulin-dependent protein kinase II inhibitor, with IC_{50} values of 3–6 μM (Ginnan *et al.*, 2004). NS1643, on the other hand, has been shown to be a more specific hERG activator, with an EC_{50} of 15 μM (Casis *et al.*, 2006), although our current study showed a 55% increase at 3 μM .

In this study, in isolated Langendorff-perfused hearts, both mallotoxin and NS1643 shortened APD and the QT interval. These two compounds also consistently increased Tp-Te, rTp-Te and coronary flow (by a presently unknown mechanism). Furthermore, the shortening of APD and QT interval by these two compounds was associated with a pro-arrhythmic potential, demonstrated by the very high incidence of VF (in all seven hearts at a concentration of 1 μM with mallotoxin, and in four out of the six hearts with NS1643 at 10 μM) in the isolated rabbit heart model. In contrast to reliable increases in Tp-Te and some degree of QT and APD shortening, the incidence of VF did not consistently correlate with beat-to-beat instability of the action potential. Indeed, although the incidence of VF (a measure of proarrhythmic potential) after NS1643 is similar to that of mallotoxin and levromakalim, beat-to-beat instability only marginally increased before VF in the presence of NS1643, whereas this parameter was markedly increased after mallotoxin and levromakalim. The reason for this difference has not been addressed in this study.

Physiological shortening of the QT interval, such as that caused by increasing the heart rate, is of course not normally

proarrhythmic. In addition, increases in body temperature markedly shorten the QT interval, without causing arrhythmia in healthy dogs (Van der Linde, personal communication). However, the recently identified congenital short QT syndromes clearly show the risks of QT shortening, and our experimental demonstration of the potential proarrhythmic risks of various QT-shortening drugs suggests that inappropriate drug-induced QT shortening can have dangers. As discussed above, although biomarkers such as beat-to-beat instability do not reliably predict the incidence of VF under these conditions, Tp-Te and rTp-Te are consistently increased in the setting of VF. Further work is required to identify both the degree of QT shortening and coincidental biomarkers that could be used to identify the risk of proarrhythmia in developmental compounds.

Limitations

The compounds tested in the hERG and APD assays in this study represent a selection of (mainly) bioactive compounds derived from Johnson & Johnson's current research projects, and, although they are chemically heterogeneous, they may not be representative of compounds tested by other pharmaceutical companies. Nevertheless, it is increasingly recognized that a large percentage of biologically active compounds will inhibit the hERG current and may affect other ion channels (Kang *et al.*, 2004; Martin *et al.*, 2004; Christ *et al.*, 2008). We therefore believe that the results obtained in this study, where 58% of compounds tested showed significant hERG inhibition, are representative of present-day medicinal chemistry.

Although drug-induced VT and VF with short QT were related to a shortening in QT or APD, exactly which biomarkers are predictive of drug-induced short QT remains unknown. In contrast to acquired long QT (for example, Hondeghem *et al.*, 2003; Lawrence *et al.*, 2006), our data in isolated hearts suggest that beat-to-beat instability did not appear to play a major role in drug-induced VF in acquired short QT. The common reason for susceptibility to malignant ventricular tachyarrhythmias in short QT is likely the large dispersion of ventricular refractoriness caused by the inhomogeneous abbreviation of the action potential within the ventricle and within different layers of the ventricular wall (Gaita *et al.*, 2003; Borggrefe *et al.*, 2005). However, although it is difficult to measure the dispersion in different layers of the ventricle, we were able to show that mallotoxin and the two K_{ATP}^+ channel openers tested significantly increased ventricular transmural dispersion taken from the transmural ECG (Tp-Te and rTp-Te). Indeed, these increases in dispersion were secondary to the significant shortening in QT/APD. Additional studies are needed to determine which additional parameters may play an important role in drug-induced short QT and VT/VF. One of the possibility may be the wavelength, λ , defined as conduction velocity \times effective refractory period ($(\text{ERP}) = \text{ERP}/\text{CT}$) and it may have a key role in predicting drug-induced VT/VF associated with short QT (see Hondeghem, 2006). When λ decreases by shortening QT and APD or slowing the conduction velocity, reentry arrhythmias such as VT and VF are most likely to occur (Girouard and

Rosenbaum, 2001). In this study, it is unknown if λ plays a crucial role in drug-induced short QT because we did not measure effective refractory period. Further studies are needed to determine the importance of λ in drug-induced short QT.

Conclusions

Although the current ICHS7B regulatory guidelines (ICH S7B, 2005) may be useful for predicting drug-induced QT prolongation, amendments to these guidelines may be warranted in the future to address the potential liability for drug-induced short QT and the associated fatal arrhythmias.

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Conflict of interest

The authors state no conflict of interest.

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