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Review

Acquired QT interval prolongation and HERG: implications for drug discovery and development

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Abstract

Putative interactions between the Human *Ether-a-go-go* Related Gene (HERG), QT interval prolongation and Torsades de Pointes (TdP) are now integral components of any discussion on drug safety. HERG encodes for the inwardly rectifying potassium channel (I_{Kr}), which is essential to the maintenance of normal cardiac function. HERG channel mutations are responsible for one form of familial long QT syndrome, a potentially deadly inherited cardiac disorder associated with TdP. Moreover, drug-induced (acquired) QT interval prolongation has been associated with an increase in the incidence of sudden unexplained deaths, with HERG inhibition implicated as the underlying cause. Subsequently, a number of non-cardiovascular drugs which induce QT interval prolongation and/or TdP have been withdrawn. However, a definitive link between HERG, QT interval prolongation and arrhythmogenesis has not been established. Nevertheless, this area is subject to ever increasing regulatory scrutiny. Here we review the relationship between HERG, long QT syndrome and TdP, together with a summary of the associated regulatory issues, and developments in pre-clinical screening.

Keywords: Long QT syndrome; Iatrogenic disease; HERG protein; Torsades de Pointes; Drug discovery; High-throughput screening

Contents

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1. The cardiac action potential

Surface recordings made during the electrocardiogram (ECG) reflect the electrophysiological events occurring during impulse generation and conduction in the heart. Each heartbeat starts as an electrical excitation generated in the sinoatrial node and is rapidly conducted throughout the atria. On surface ECG measurements, the 'P' wave represents the combined electrical activity of action potential depolarisation in the atria (Fig. 1A). Impulse conduction to the ventricles occurs though the atrioventricular node and is transmitted rapidly across both ventricles via the His–Purkinje system, by virtue of tight electrical coupling between ventricular cells. The QRS complex of the ECG corresponds to the action potential depolarisation as it occurs in the ventricles (masking the electrical activity associated with action potential repolarisation in the atria), while the T wave is associated with ventricular repolarisation (Fig. 1A). Thus the QT interval of an ECG represents the duration of the ventricular action potential, plus the time associated with transmission across the myocardium (Belardinelli et al., 2003). It therefore follows that a prolongation of the QT interval corresponds to a prolongation of the ventricular action potential (Fig. 1A). In humans, the ventricular action potential is typically 200–300 milliseconds (ms) in duration. Prolongation of a heart-rate corrected (QT_c) interval[1] in excess of ~440 ms for men or ~460 ms for women has for sometime been associated with increased mortality (Schouten et al., 1991).

The action potential of all ventricular cells shares many ionic and electrical similarities, although variation exists between species, region and function (Haverkamp et al., 2000). The typical mid-myocardial ventricular action potential is shown schematically (Fig. 1B). Initially, the cell is polarised near to the electrochemical potential for potassium ions (E_K) because of high K^+ conductance at rest. A rapid depolarisation, mediated by a Na^+ current, and not blocked by tetrodotoxin in many species, is followed by a brief partial repolarisation mediated by a transient outward current (I_{to}), consisting of two components, one a Cl^- current, and the other by a K^+ current. I_{to} is the principal repolarising current of the ventricular action potential in mice and rats, which have very fast heart rates. However, in

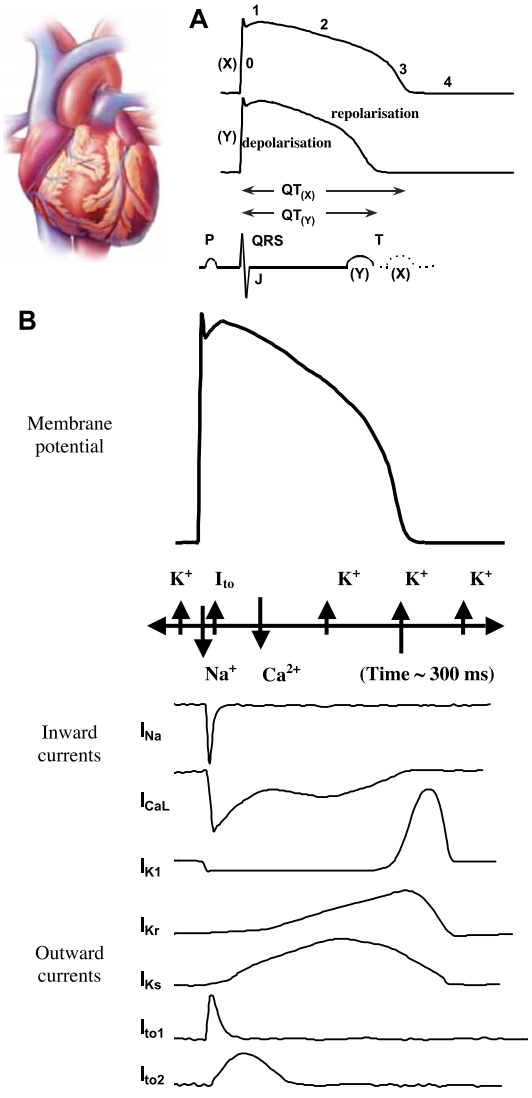

Fig. 1. In panel A, the QT interval correlates with the cardiac ventricular action potential duration. The QRS time course plus the JT interval represents the absolute QT interval. In the schematic representation, the ventricular action potential of (X) is prolonged compared to (Y). The upper action potential is labelled with its respective phases including rapid upstroke (0), early repolarisation (1), plateau phase (2), late repolarisation (3) and post repolarisation. The diagrammatic representation of the ECG shows the timing of $QT_{(X)}$ and $QT_{(Y)}$. Panel B shows the membrane voltage profile of a ventricular action potential in association with ion movement across the myocyte membrane and the major (not all for simplicity) currents contributing the action potential over time (300 ms). Inward (downward) currents contribute to depolarisation as shown in panel (A), and outward (upward) to repolarisation.

[1] QTc is defined as the QT interval corrected for heart rate. Several methods (>30) for calculating QTc exist, although the most common is Bazett (Shah, 2002).

humans, and other species used for studying cardiac cellular electrophysiology such as dogs, guinea pigs, rabbits, swine and ferrets, I_{to} is not the principal ventricular repolarising current (Crumb and Cavero, 1999). The plateau phase of the action potential then follows; this is a dynamic equilibrium between the movement of Ca^{2+} into and K^+ out of the cell. As K^+ efflux exceeds Ca^{2+} influx, the cell membrane slowly repolarises. This repolarisation reduces the Ca^{2+} current which, augmented by an increase in conductance of some K^+ channels, results in a positive feedback loop. In the final stage of the action potential, the rapid repolarisation phase, the augmenting K^+ current rapidly induces complete repolarisation, thereby restoring the original resting potential of the myocyte (Fig. 1B).

The rate of K^+ efflux, and by extension the rate of repolarisation, is determined by the density and gating properties of different K^+ channels. Distinct K^+ channels feature in the initiation and completion of action potential repolarisation. The delayed rectifier current (I_K), comprising both rapid (I_{Kr}) and slow (I_{Ks}) components, is particularly important in regulating the duration of the action potential plateau (Fig. 1B). Composite I_K develops progressively during the plateau phase, opposing the inward currents underlying plateau depolarisation. Slowing of action potential repolarisation can lead to the generation of spontaneous depolarisations on the falling phase of the action potential plateau. Unlike the action potential itself, these events, termed 'Early After Depolarisations', are not synchronised with an excitatory stimulus, and so can give rise to asynchronous tissue excitation and thereby arrhythmogenesis.

Under physiological conditions, the action potential progresses through the phases of depolarisation, plateau phase and repolarisation over the course of 200–300 ms; however, in clinical conditions such as long QT syndrome, repolarisation is delayed and the action potential duration extended. Prolongation of the action potential could theoretically arise from an increase in inward (depolarising) current or from a reduction in outward (repolarising) current, both of which exist in different populations of long QT syndrome patients.

2. Familial and acquired long QT syndrome

Long QT syndrome exemplifies one of the best understood cardiac disorders. "Long QT syndrome" was originally coined by Jervell and Lange-Nielsen in 1957 who observed an association between prolongation of the QT interval of the ECG with syncope and sudden death. These symptoms are now intrinsically linked with Torsades de Pointes (TdP; Dessertenne, 1966), a distinctive polymorphic ventricular tachycardia (with concomitant QTc prolongation), characterised by continuously alternating QRS complexes, which can degenerate into ventricular fibrillation, ultimately leading to sudden death in around 20% of untreated patients (Shah, 2002). Familial long QT syndrome is the inherited form of this cardiac disorder (Ward, 1964; Romano, 1965), which affects young (mean age of 24 years, Moss et al., 1985), otherwise healthy individuals and is characterised by early symptom onset, frequent, recurrent, non-sustained tachyarrhythmia's, and a labile, prolonged QT interval on the ECG (Vincent et al., 1992). In a large prospective study, Moss et al. (1991) reported that patients diagnosed as having long QT syndrome had a higher frequency of syncope (fainting/collapse) or cardiac arrest with resuscitation (80%), a resting heart rate of less than 60 beats/min (31%), a history of ventricular tachyarrhythmia (47%), and a higher rate of congenital deafness (7%) than unaffected family members. The arrhythmogenic syncope caused by TdP is often associated with acute physical, emotional, or auditory arousal. Indeed, literature relating to the variant of the disorder associated with congenital deafness pre-dates the development of the ECG. Meissner (1856) described a deaf girl who collapsed and died while being admonished at school. The child had two brothers who also died suddenly after violent fright or rage. It was more than a century later when Jervell and Lange-Nielsen (1957) presented the electrocardiographic basis for long QT syndrome when they described four deaf children with QT interval prolongation, three of whom died suddenly. Thereafter, reports of long QT syndrome without deafness, which were clearly distinct from the recessively inherited Jervell and Lange-Nielsen syndrome, appeared (Ward, 1964). Descriptions of families involving multiple generations of long QT syndrome sufferers reported around the same time (Barlow et al., 1964; Romano, 1965; Garza et al., 1970), pointed to a higher incidence than originally thought.

Insight into the electrophysiological causes underlying long QT syndrome has come from genetic studies, with linkage analysis now showing that of the seven genes associated with familial long QT syndrome, six are ion channel subunits expressed in the heart (Table 1). The first of these genes to be associated with long QT syndrome was the Human *Ether-a-go-go* Related Gene (HERG), a voltage-gated potassium channel alpha subunit that mediates the rapid component of the delayed rectifier

Table 1
Defects in familial long QT syndrome (LQT)

LQT (RW)	Current	Chromosome	Gene	Channel
LQT1	I_{Ks}	11p15	*KCNQ1*	KvLQT1
LQT2	I_{Kr}	7q35	*KCNH2*	HERG
LQT3	I_{Na}	3p21	*SCN5A*	–
LQT4	?	4q25	*ANKB*	ANK2
LQT5	I_{Ks}	21q22	*KCNE1*	minK
LQT6	I_{Kr}	21q22	*KCNE2*	MiRP1
LQT7	I_{K1}	17q23	*KCNJ2*	Kir 2.1
JLN	I_{Ks}	11p15	*KCNQ1*	KvLQT1
	I_{Ks}	21q22	*KCNE1*	minK

RW is Romano–Ward and JLN is Jervell and Lange-Nielsen.

potassium current, I_{Kr} (Curran et al., 1995; Sanguinetti et al., 1995). In addition to HERG (also known as KCNH2 or $K_v11.1$), others channels including a Na^+ channel called SCN5A ($Na_V1.5$; Wang et al., 1995), a previously unidentified K^+ channel called KvLQT1 (KCNQ1 or $K_V7.1$; Barhanin et al., 1996; Sanguinetti et al., 1996), and two putative K^+ channel beta subunits, KCNE1 (minK; Splawski et al., 1997) and KCNE2 (hMiRP1; Abbott et al., 1999) have been identified (Table 1). Most cases of familial long QT syndrome are caused by defects in expression or regulation of ion channels controlling electrical activity in ventricular cells. Even though the prevalence of familial long QT syndrome is less than 1/100,000 (Puddu et al., 2001), in excess of 177 distinct mutations have now been found in long QT syndrome patients (Walker et al., 2003).

When HERG was first demonstrated to be the K^+ channel responsible for chromosome-7-associated (LQT2) *familial* long QT syndrome (Curran et al., 1995), it was also suggested that pharmacological inhibition of HERG was a possible mechanism for *acquired* long QT syndrome (Sanguinetti et al., 1995); acquired long QT syndrome affects a much older patient group and is associated with administration of drugs that produce QT prolongation. The subsequent finding that mutations in the gene encoding I_{Ks} were involved in a different form of familial long QT syndrome (LQT1; Wang et al., 1996) raised the possibility that pharmacological inhibition of I_{Ks} might also produce, or contribute to, acquired long QT syndrome. However, while the role of I_{Ks} in human cardiac action potential repolarisation has been questioned (Crumb and Cavero, 1999), it cannot yet be excluded (Redfern et al., 2003). However, as discussed below (Redfern et al., 2003), HERG/I_{Kr} has become intrinsically associated with acquired long QT syndrome and by analogy TdP.

3. Human *Ether-a-go-go* Related Gene (HERG)

HERG was originally cloned by homology screening from a human hippocampal library (Warmke and Ganetzky, 1994). In other species, transcripts are expressed strongly in brain, slightly less so in heart, testis and lung, with much lower expression found in skeletal muscle, adrenal gland, and thymus (Wymore et al., 1997). The putative structure of the HERG channel (1159 amino acids) reveals HERG to be similar in many respects to other members of the shaker-type voltage-gated K^+ channel families (Warmke and Ganetzky, 1994). These K^+ channels are made up of four subunits (Mackinnon, 1991), each of which has six α-helical transmembrane domains and a looping "pore region" (Papazian et al., 1987). The transmembrane domains are functionally organised such that S5 and S6 and the looping pore region contribute to the pore, and the S4 region includes regularly spaced charged amino acids which function as the voltage sensor (Isacoff et al., 1990). The HERG channel has N- and C-termini which lie intracellularily, with the former functionally involved in channel deactivation (Schonherr and Heinemann, 1996). X-ray crystallography has shown the structure of the N-terminus to be related to a Per-Arnt-Sim regulatory domain (Morais Cabral et al., 1998).

As discussed above, I_{Kr} encoded by HERG is one of the major currents responsible for repolarisation of the cardiac myocyte ventricular action potential (Sanguinetti and Keating, 1997). HERG and I_{Kr} (Sanguinetti et al., 1995) are pharmacologically similar, being blocked by methanesulphonanilide class III antiarrhythmics, such as 4′ -[[1-[2-(6-methyl-2-pyridyl)ethyl]-4-piperidyl]carbonyl]-methanesulfonanilide (E-4031), (+)-*N*-[1′ -(6-cyano-1,2,3,4-tetrahydro-2(*R*)-naphthalenyl)-3,4-dihydro-4(*R*)-hydroxy-spiro(2*H*-1-benzopyran-2,4′ -piperidin)-6-yl]methane-sulfonamide monohydrochloride (MK-499) and dofetilide (Sanguinetti and Keating, 1997). The unique kinetics of the HERG channel (inactivation faster than activation) means that relatively little current flows at the peak of the action potential overshoot, with the channels rendered non-conducting by rapid inactivation (Hancox et al., 1998; Zhou et al., 1998). This means that during a ventricular action potential, the channel current increases greatly during the repolarisation phase as inactivation is removed (Fig. 1). The inhibition of I_{Kr} leads to excessive lengthening of the action potential, which can induce early after depolarisations (leading to ectopic beats), and along with increased dispersion of ventricular repolarisation, probably underlies the cellular mechanism of TdP (Sanguinetti and Keating, 1997; Belardinelli et al., 2003). The link between HERG/I_{Kr} acquired QT interval prolongation and TdP has become one of the foremost safety issues in drug development (Fermini and Fossa, 2003; Morganroth, 2004). With ever increasing regulatory scrutiny, any association of new chemical entities with these issues might adversely effect drug development (Crumb and Cavero, 1999). The need to identify high-throughput screens that can be used early in pre-clinical drug development to assess the potential for prolonged ventricular repolarisation is paramount. Below, we discuss the current regulatory position, and the development and use of new and old assays that may directly or indirectly detect compounds with arrhythmogenic potential at the pre-clinical stage.

4. Acquired QT interval prolongation and regulation; from CPMP to ICH S7B

In the first half of the 1990s, non-cardiovascular drugs such as astemizole, cisapride, terfenadine and pimozide were found to cause QT interval prolongation and TdP (see Shah, 2002). While none of these drugs were used for life threatening diseases, all could potentially have been

prescribed on a long-term basis. In response to these observations, the Committee for Proprietary Medicinal Products (CPMP) of the European Union issued in 1997 'a points to consider' document on the assessment of the potential for QT interval prolongation by non-cardiovascular medicinal products (http://www.emea.eu.int/pdfs/human/swp/098696en.pdf). The impact of these 'guidelines', on drug discovery and development has been, and will be for the foreseeable future, extremely significant. It appears that Public Health Authorities now expect all new chemical entities to be tested in cardiac electrophysiological studies (Haverkamp et al., 2000; Shah, 2002). QT interval prolongation (and I_{Kr} block) which is regarded as a surrogate for Torsades-type dysrrhythmias is still not well understood; however, it has become the number one safety issue in the development of pharmaceuticals, superseding liver injury in being the primary cause of drug withdrawals in the last decade (Shah, 2002; Fossa et al., 2004; Morganroth, 2004). Such interest culminated in the First International Internet Symposium on Long QT Syndrome taking place in April 2004 (http://lqts-symposium.org). The number of drugs associated with TdP continues to increase (http://torsades.org), despite the incidence of drug-induced TdP being less than 1/100,000 (Haverkamp et al., 2000; Darpo, 2001; Redfern et al., 2003; Morganroth, 2004). The CPMP document was followed by the Canadian draft in 2001, the Health Canada/USA draft in 2002 and the on-going International Conference on Harmonisation (ICH) S7B (pre-clinical) and E14 (clinical) documents discussed below (Fermini and Fossa, 2003; Morganroth, 2004).

Almost all drugs that induce TdP in humans block I_{Kr} and cause QT interval prolongation; however, the converse is not always the case. Indeed, development of TdP is quite unpredictable (Yang et al., 2002; Witchel et al., 2003; Fossa et al., 2004). In addition to the estimated clinical incidence of TdP being low (Haverkamp et al., 2000; Darpo, 2001; De Ponti et al., 2002; Morganroth, 2004), this arrhythmia is often self-terminating, with degeneration in to ventricular fibrillation less frequent (Shah, 2002; Belardinelli et al., 2003). Epidemiological studies have, in general, failed to demonstrate an increased risk of proarrhythmia for non-cardiovascular drugs (Darpo, 2001). Moreover, the use of drugs known to cause QT interval prolongation is not always associated with an increase in frequency of ventricular arrhythmias (De Ponti et al., 2000, 2002). Although a positive result in pre-clinical and/or clinical studies should not preclude continued clinical development (Shah, 2002), there does appear to be a perception that problems will surround any drug associated with QT interval prolongation (Haverkamp et al., 2000; Webster et al., 2002; Pearlstein et al., 2003). While delayed repolarisation per se is not proarrhythmic (Belardinelli et al., 2003), acquired QT interval prolongation is no longer a 'pharmacological curiosity' (De Ponti et al., 2000).

4.1. CPMP/986/96: points to consider: the assessment of the potential for QT interval prolongation by non-cardiovascular medicinal products—pre-clinical studies

The perceived association between medicinal products that prolonged the cardiac action potential and 'torsades de pointes' underlay the formation of an expert working group to address pre-clinical and clinical testing of such compounds. The expert group highlighted the potential ramifications for non-cardiac medicinal products (antibiotics, histamine H_1 receptor antagonists and psychotropic agents) producing QT interval prolongation/TdP, and the implications for future drug development. Pre-clinical studies suggested at that time included in vivo cardiovascular safety pharmacological studies, usually in the dog, and in vitro electrophysiological studies (papillary muscle and Purkinje fibres) on an appropriate species (Crumb and Cavero, 1999). The expert working group also identified various risk factors that could predispose individuals to a proarrhythmic episode. These included cardiac disease ($\downarrow I_{Kr}$, $\uparrow$ action potential duration), familial long QT syndrome, hypokalaemia ($\downarrow I_{Kr}$, $\uparrow$ action potential duration), hypomagnesia, bradycardia ($\uparrow$ action potential duration) and concurrent administration of other medicinal products known to cause QT interval prolongation. In addition, female gender (longer QT interval), rapid drug administration (i.v.) and age ($\uparrow$ action potential duration) are considered as predisposing factors for TdP (Haverkamp et al., 2000; Bode and Olejniczak, 2002; Redfern et al., 2003).

4.2. ICH S7B: safety pharmacology studies for assessing the potential for delayed ventricular repolarisation (QT interval prolongation) by human pharmaceuticals

An expert working group was tasked with generating a second safety pharmacology guideline, ICH S7B (http://www.emea.eu.int/pdfs/human/ich/042302.pdf; Bode and Olejniczak, 2002) specifically addressing QT interval prolongation, extending and complementing ICH S7A (http://www.emea.eu.int/pdfs/human/ich/053900en.pdf; Wakefield et al., 2002). Although released for consultation in February 2002, with projected implementation in 2004, the guideline is currently still in draft form (at the time of writing: March 2004), with no definitive date set for moving to the next step in the ICH process (see ICH6 presentations on Safety and Efficacy; http://www.ich.org; Bode and Olejniczak, 2002). The relationship between ICH S7B and the as yet unpublished ICH E14 (The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs; Fermini and Fossa, 2003; Morganroth, 2004) is uncertain. The complexity of the issues being addressed calls in to question whether non-clinical studies are likely to be prognostic (Morganroth, 2004), despite this assertion in the original CPMP document (Webster et al., 2002) and previous studies (Davis, 1998). It has been suggested that

ICH E14 may contain a recommendation that a 'thorough QT/QTc study' should "almost always" be carried out, irrespective of non-clinical data, although this position is not definite (ICH6, Safety and Efficacy; http://www.ich.org; Morganroth, 2004). However, two recent industry-sponsored initiatives (ILSI/HESI and QT PRODACT) suggest that non-clinical data may indeed be useful for identifying compounds associated with TdP in humans (K. Fujimori and PKS. Siegl—ICH6; http://www.ich.org). These initiatives appear to favour a primary ('core') of non-clinical tests that includes an in vitro I_{Kr} assay (HERG) and an in vivo QT assay. Data from studies on action potential duration were variable and as such may be used in a follow-up capacity (http://www.ich.org), rather than as an integral assessment as originally outlined. These results are consistent with earlier observations by Hondeghem et al. (2001), which questioned the use of action potential duration measurements as a surrogate marker for proarrhythmia.

The issue of 'what is a safe drug' has been reviewed recently by Redfern et al. (2003), who compared pre-clinical and clinical data for 100 compounds from antiarrhythmics through to drugs with no known association with TdP. Although evidence was provided for a putative safety margin, in line with previous suggestions (Webster et al., 2002), regulatory concern over interpreting clinical safety margins from in vitro data may underlie the absence of definitive numbers in later drafts of ICH S7B. In the past 5 years, many schematics have been presented on what, and when, studies should be performed to identify compounds with a propensity to induce QT interval prolongation and or TdP (Crumb and Cavero, 1999; Haverkamp et al., 2000; Netzer et al., 2001; De Ponti et al., 2002; Bode and Olejniczak, 2002; Fermini and Fossa, 2003). However, it is not inconceivable that in exclusively addressing QT interval prolongation, both clinically and pre-clinically, we are failing to examine whether compounds will actually cause TdP, which is ultimately the primary issue (Antzelevitch and Shimizu, 2002; Hondeghem et al., 2001, 2003; Belardinelli et al., 2003; Di Diego et al., 2003; Witchel et al., 2003). However, the present inability to separate safe from torsadogenic HERG blockers leaves Regulatory Authorities with little option (Fermini and Fossa, 2003). Nevertheless, it is important to recognise that the correlation between drug-induced TdP and the length of QT interval prolongation is weak (Darpo, 2001; Belardinelli et al., 2003; Redfern et al., 2003).

Many factors, other than heart rate, are known to produce large alterations in QTc. These include age (3–6 ms/10 years; Magnoni et al., 2003), gender and diurnal rhythm (23 and 95 ms, respectively; Molnar et al., 1996), posture (7 ms; Davey, 1999) and even space flight (20–70 ms; D'Aunno et al., 2003). Indeed, Paltoo et al. (2001) have reported a case of polymorphic ventricular tachycardia in the absence of QT interval prolongation. The key factors required for drug-induced TdP in humans and animals appears to be transmural dispersion of ventricular repolarisation, early after depolarisations and the induction of ectopic beats (Belardinelli et al., 2003), although the precise relationship is not clear. QT interval prolongation alone is not wholly predictive, nor sufficient to induce TdP (Malik and Camm, 2001; Belardinelli et al., 2003). It is vital that a distinction, if possible, be made between acquired and spontaneous QT interval prolongation (Haverkamp et al., 2000). At a clinical level, hypothyroidism, chronic amiodarone administration, and severe hypocalcaemia produce considerable QT interval prolongation, but are rarely associated with TdP unless there are concomitant electrolyte disturbances. A common feature of all three conditions is a marked inhibition of L-type Ca^{2+} currents (I_{CaL}). Moreover, the use of magnesium therapy, which may attenuate Ca^{2+} influx (Wu and Lipsius, 1990), is efficient at abolishing arrhythmia in clinical TdP and in animal models, although not fully normalising the QT interval (Arsenian, 1993). Simultaneous inhibition of I_{CaL} may therefore reduce the arrhythmogenic risk associated with HERG blockade or QT interval prolongation. Citalopram may be an example of this dual effect. Initially, data concerning self-poisonings had raised the possibility that citalopram (at high concentrations) might lead to a risk of arrhythmia, although this was put into perspective with regard to other risks for these patients (Personne et al., 1997). Citalopram blocked HERG at concentrations similar to that observed for other antidepressants, although this selective serotonin re-uptake inhibitor also blocked I_{CaL} (Witchel et al., 2002b). This protective effect of I_{CaL} blockade may explain the low fatal toxicity index scores associated with selective serotonin re-uptake inhibitors (Henry, 1997).

A simple analysis of compounds associated with I_{Kr} inhibition, QT interval prolongation and TdP (torsades.org website, categories 1–3; De Ponti et al. (2002) and http://www.fenichel.net) reveals that of the 214 compounds cited, 139 (65%) are in clinical use in the UK (Table 2). Such associations do not however make safe drugs become suddenly unsafe (Redfern et al., 2003). As to whether acquired QT interval prolongation and TdP is occurring more frequently with new drugs (Di Diego et al., 2003) is difficult to truly assess. Episodes of quinidine-induced syncopy date back to the 1920s (Walker et al., 2003), and reports of cardiac arrhythmias associated with psychotropic drugs have appeared in the literature for the last 40 years (Witchel et al., 2003). Whether the apparent increase in frequency of acquired QT interval prolongation in the last decade is entirely due to improvements in safety monitoring is unclear. Moreover, the concept that familial and acquired long QT syndrome is clinically distinct with different modes of initiation of TdP is questionable in light of reports connecting the two conditions (Walker et al., 2003). Zehender et al. (1991) reported that QT interval prolongation was observed in 56–71% of patients prior to drug-induced proarrhythmia indicating abnormalities in repolarisation were already present. In addition, it is clear that some patients with acquired QT interval prolongation have

Table 2
This table categorises drugs potentially associated with I_{Kr} inhibition, QT interval prolongation and Torsades de Pointes with reference to the British National Formulary (2003)

Classification	Compound numbers (in clinical use in UK)	Number of compounds per category
Gastro-intestinal system	4 (2)	2 of 9
Cardiovascular system	60 (32)	7 of 13
Respiratory system	25 (16)	3 of 10
Central nervous system	62 (50)	10 of 11
Infections	25 (17)	4 of 5
Endocrine system	2 (2)	2 of 7
Obstetrics/gynaecology[(a)]	6 (4)	2 of 4
Malignant disease[(b)]	5 (4)	3 of 3
Nutrition and blood	None listed	0 of 8
Musculoskeletal and joint	1 (1)	1 of 3
Eye	3 (3)	2 of 9
Ear, nose and oropharynx	None listed	0 of 3
Skin	None listed	0 of 4
Immunological/vaccines	None listed	N/A
Anaesthesia	11 (8)	2 of 2

The non-clinical and clinical compounds included in the categorisation were obtained from the following three sources: the torsdades.org website (categories 1–3), De Ponti et al. (2002) and the webpage of Dr. R.R. Fenichel (http://www.fenichel.net; only compounds associated with I_{Kr} inhibition). Of the 214 compounds included, 139 or 65% are presently in clinical use in the UK. (a) includes urinary tract disorders and (b) immunosuppression.

mutations or polymorphisms in genes encoding both alpha and beta-potassium channel subunits (Walker et al., 2003). The extent to which mutations exist or play a role in acquired QT interval prolongation remains the subject of much debate (Puddu et al., 2001; Paulussen et al., 2004).

5. Methodologies for identifying HERG/I_{Kr} inhibitors in vitro

As of today, the majority of in vitro screening methodologies tend to utilise either cell lines heterologously expressing human cardiac ion channels, cardiac cell cultures, isolated tissues preparations such as Purkinje fibres and perfused heart preparations (Haverkamp et al., 2000; Webster et al., 2002). For high-throughput screening purposes, heterologous HERG expression is generally favoured over 'native' I_{Kr} currents, due to the lack of contaminating currents, increased signal sizes and comparability of data (Crumb and Cavero, 1999; Yang et al., 2001; Witchel et al., 2002a; see ICH S7B). A summary of some of the currently available and developing methodologies are described in the following sections.

5.1. In vitro electrophysiology

To date, electrophysiology remains the 'gold standard' method with which to characterise ion channel properties (Witchel et al., 2002a), as binding, flux and fluorescence assays only indirectly measure ion channel properties. In vitro and in vivo electrophysiological methodologies are extensively discussed in ICH S7B and other publications (see Haverkamp et al., 2000). Electrophysiological studies are still perceived as labour- and skill-intensive, and low throughput. However, this situation is rapidly evolving through the generation of a range of high-throughput electrophysiological systems, some of which are successfully being used to examine HERG channel function (Kiss et al., 2003). The range and source of automated planar array-based high-throughput voltage clamp systems that are/becoming available have been recently discussed by both

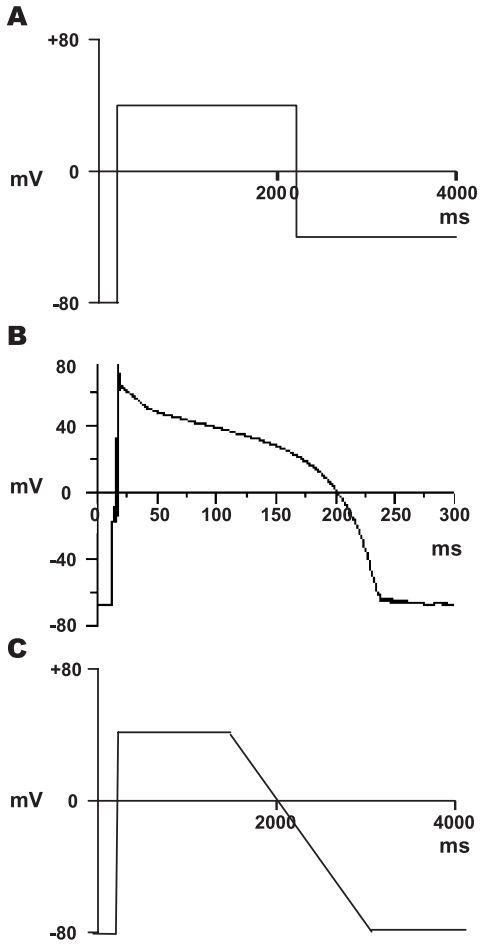

Fig. 2. Electrophysiological protocols for evaluating HERG blockade. Panel A shows a traditional square pulse voltage command protocol. Assessment of HERG blockade is performed using maximum tail currents, determined during the repolarising step (in this case to −40 mV). This protocol leads to HERG being fully activated during the initial depolarising step with the channel's inactivation characteristics dependent upon the initial depolarising voltage. Panel B describes an action potential clamp approach, in which a digitised action potential wave form is used as the voltage command protocol (Hancox et al., 1998). Assessment of HERG blockade is made at the peak current, which typically occurs between −30 and −40 mV. Choice of the source of the action potential used (e.g. epicardial or endocardial) is crucial to interpretation of the data. Panel C describes a descending ramp protocol, which represents a hybrid between a square pulse protocol and action potential clamp. This approach offers some advantages of being more physiologically relevant than the square pulse protocol and being technically simpler than the action potential clamp approach; however, it is the least common of these approaches within the HERG literature.

Bennett and Guthrie (2003), and Netzer et al. (2003). In time, these systems will undoubtedly have a major impact on ion channel drug discovery. However, despite the advent of this new technology, no clear guidelines exists on what protocols should be used to examine HERG channels in vitro. A number of different approaches are currently in use including square pulse, action potential and ramp protocols, all of which are suitable (Fig. 2). Clearly, until high-throughput electrophysiology becomes user-friendly and cost-effective, other technologies will continue to be used in the majority of laboratories.

5.2. Radioligand binding assays

Radioligand binding assays do not provide the depth of information generated in electrophysiological studies; however, they do constitute a methodology which is relatively simple, amenable to miniaturisation and accessible at reasonable cost to the majority of laboratories. For HERG, this approach was first applied when a [^{3}H]dofetilide binding assay was proposed as a primary screen for compounds which cause QT interval prolongation in man (Finlayson et al., 2001a). The use of membranes from human embryonic kidney (HEK293) cells expressing HERG (Zhou et al., 1998) minimised binding of [^{3}H]dofetilide to an endogenous site in untransfected cells (Finlayson et al., 2001b; Finlayson and Sharkey, 2004). Other binding assays have subsequently been described using [^{35}S]MK-499 (Wang et al., 2003), [^{125}I]BeKm-1 (Angelo et al., 2003) and, most recently, [^{3}H]astemizole, presented by Dr. P. Chiu at the QT Prolongation and Safety Pharmacology meeting (London, January 2004). Intriguingly, attempts to establish a binding assay using radiolabelled E-4031 were unsuccessful (P. Chiu, personal communication). In contrast to the limited number of compounds examined in the [^{35}S]MK-499 and [^{125}I]BeKm-1 binding assays, Dr. Chiu presented data on 32 compounds from different chemical classes, which has allowed us to draw a correlation between the [^{3}H]dofetilide and [^{3}H]astemizole binding assays. Analysis of 12 compounds, from seven drug classes (antiarrhythmics, antihistamines, anti-oestrogen, calcium antagonist, antipsychotics, antifungal and antidepressants), revealed an excellent correlation (r^2=0.955) in binding affinities using these two radioligands (Fig. 3).

While simple to perform, HERG radioligand binding assays cannot discriminate between agonists and antagonists. Furthermore, the likelihood of all compounds binding at the same site on the HERG channel as the radioligand has been questioned (Netzer et al., 2001; Fermini and Fossa, 2003; see ICH S7B). However, the success of the [^{3}H]dofetilide/[^{3}H]astemizole binding assays probably stems from the observation that the majority of drugs act at the intracellular region of the pore (Mitcheson and Perry, 2003; Pearlstein et al., 2003). The situation is complex and factors such as lipophilicity need to be considered (Friesen et al., 2003; Fraley et al., 2004). Clarithromycin which blocks HERG extracellularly also inhibits [^{3}H]dofetilide binding (Volberg et al., 2002). The combination of intracellular ([^{3}H]dofetilide; Finlayson et al., 2001a), extracellular ([^{125}I]BeKm-1; Angelo et al., 2003; Milnes et al., 2003) and closed state (ergotoxin or APETx1; Pardo-Lopez et al., 2002; Diochot et al., 2003) HERG channel blockers may facilitate the detection of all classes of HERG blocker.

5.3. Rubidium flux assays

For over quarter of a century, isotopic ion flux assays have been used to characterise the functional properties of voltage -or ligand-gated ion channels (Lukas et al., 2002). Radioactive rubidium (^{86}Rb) has been extensively used to study potassium channels because of its high permeability (Gill et al., 2003). Reluctance by sections of the scientific community to use this highly radioactive compound has been largely overcome by the development of a non-radioactive rubidium flux assay, quantified using atomic absorbance spectrometry (AAS; Terstappen, 1999; Scott et al., 2003). Both methodologies have been applied to studying HERG, with varying degrees of success (Tang et al., 2001; Cheng et al., 2002; Gill et al., 2003). Assay conditions have a considerable effect on antagonist potency, resulting in both methods producing lower IC_{50} values than patch clamp or binding studies (Tang et al., 2001; Cheng et al., 2002; Gill et al., 2003). Furthermore, while AAS avoids the use of radioactivity, it is still only regarded as medium throughput (Netzer et al., 2003; Scott et al., 2003).

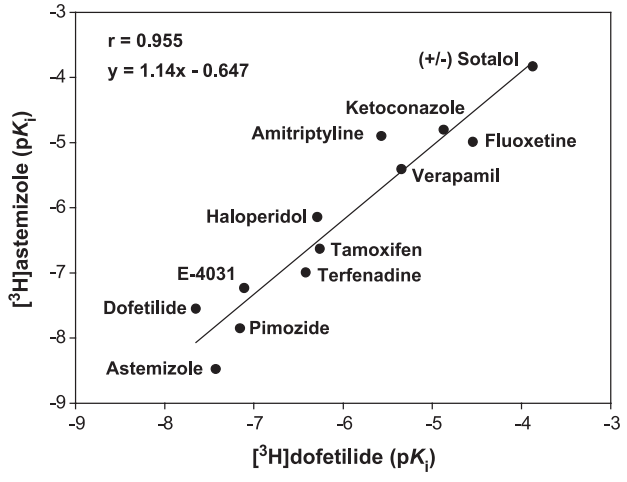

Fig. 3. Correlation of binding affinities (pK_i) for a range of HERG channel blockers in [^{3}H]dofetilide and [^{3}H]astemizole binding assays. For [^{3}H]dofetilide binding, pK_i values were determined using HERG transfected membranes as previously described (Finlayson et al., 2001a). [^{3}H]Astemizole (1.5 nM; 60 min at 25 °C) binding was also conducted using HEK293-HERG transfected membranes (Chiu et al., submitted for publication). Data shown are mean values from more than three independent experiments in both assays.

5.4. Fluorescence-based assays

The membrane potential of a cell is dependent upon the electrochemical gradient across the cell and the permeability of the membrane to individual ions (Hancox et al., 2000). Membrane permeability is therefore regulated by the presence of distinct ion channels and changes in membrane potential occur as a consequence of altering the conductance of single or multiple channels; thus as for flux assays, changes in membrane potential are a indirect measurement of channel activity. Alterations in membrane potential have historically been monitored using voltage-sensitive dyes like $DiBAC_4(3)$, or more recently the FLIPR membrane potential (FMP) dye (Baxter et al., 2002). Tang et al. (2001) compared both dyes in KCl-stimulated CHO-K1 cells expressing HERG, using different channel blockers. Although a better correlation was obtained between patch clamp data and studies using the FMP dye, neither dye appeared ideal (Tang et al., 2001). We have used the FMP dye and HEK293 cells expressing HERG in an attempt to correlate functional data with the [^{3}H]dofetilide binding assay. Unlike Tang et al. (2001), we were unable to use a KCl stimulus, as during the pre-incubation period, drugs such as dofetilide produced a concentration-dependent increase in fluorescence with a half maximal value of 540±62.5 nM (n_H=0.96±0.08, n=6; Fig. 4). The potency of dofetilide is approximately 10-fold less than in binding studies (Finlayson et al., 2001a; Finlayson and Sharkey, 2004), as expected for fluorescent-based assays (Fermini and Fossa, 2003). In contrast, other compounds such as the antihistamine terfenadine had no effect on basal fluorescence (Finlayson, unpublished observations). This effect was not observed in a CHO-K1 cell line stably expressing HERG; however, this may reflect lower channel expression and a difference in resting membrane potential, as EVOTEC OAI (Germany) have successfully exploited a similar approach using a CHO-HERG cell line (Netzer et al., 2001, 2003). FRET-based voltage-sensing dyes, although potentially useful, have similar problems to the membrane potential dyes and have not yet been reported for use with HERG (Bennett and Guthrie, 2003; Gill et al., 2003).

Ultimately, the cardiac action potential is a composite of different ion channel conductances, with prolongation potentially produced by alterations in Na^+, Ca^{2+} or K^+ currents (Bode and Olejniczak, 2002; Di Diego et al., 2003). This underscores the 'whole heart on a plate' concept, whereby some service providers have developed technologies to screen for an interaction with many different cardiac ion channels. In summary, the use of fluorescent dyes for the study of HERG is still in its infancy and, as noted in the recent ICH S7B discussion document, "more experience is necessary to establish whether they have sufficient predictive value to be an alternative to voltage clamp assays".

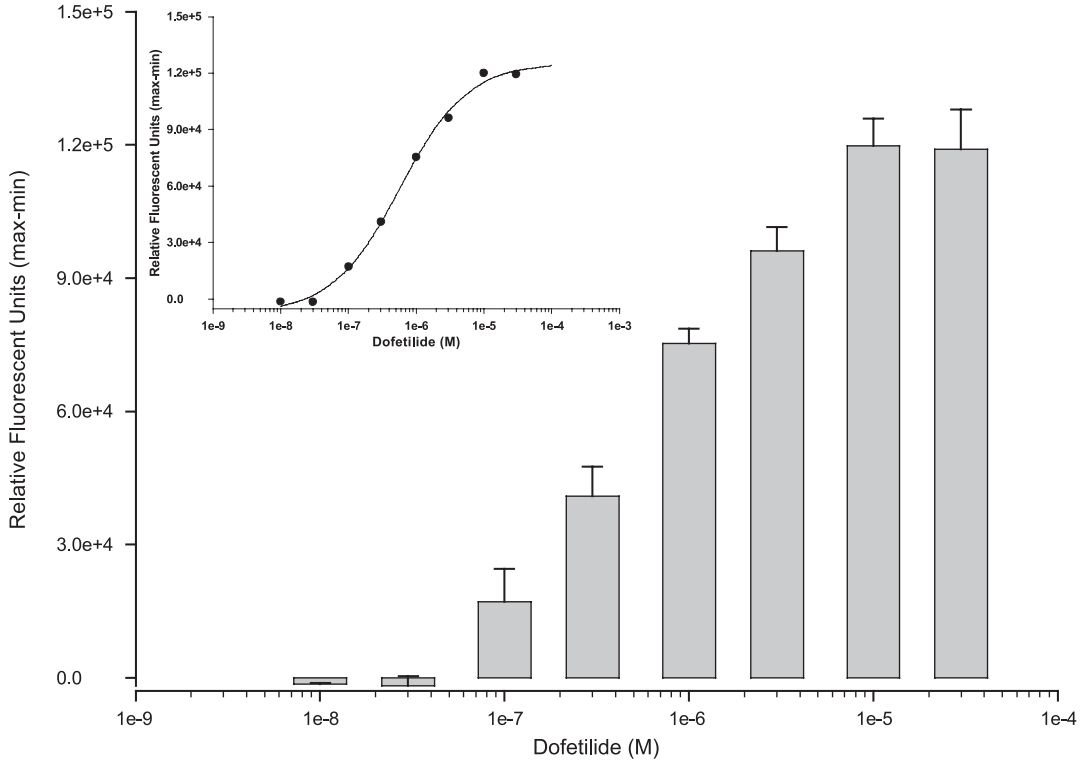

Fig. 4. Dofetilide induced a concentration-dependent increase in fluorescence in HERG transfected cells. HEK293 cells stably expressing HERG were plated at 1×10^5 cells/well and left overnight. Cells were loaded with Molecular Devices FMP dye for 30 min, and fluorescence measured using the FlexStation. Dofetilide produced a concentration-dependent increase in fluorescence over time (data shown after 90 s), with a half-maximal value of 539.6±62.5 nM.

5.5. In silico modelling of HERG

The last 3 years have seen considerable progress in defining structure–activity relationships for HERG (Mitcheson et al., 2000; Cavalli et al., 2002; Ekins et al., 2002; Roche et al., 2002; Keseru, 2003; Mitcheson and Perry, 2003; Pearlstein et al., 2003), which may help minimise channel inhibition. The original structural studies described by Mitcheson et al. (2000) were based on the KcsA crystal structure (Doyle et al., 1998), which is representative of a closed K^+ channel structure (Jiang et al., 2002); however, the recent crystallisation of the open K^+ channel conformation (MthK) and the complete structure of K_vAP will undoubtedly facilitate studies of ion channel function (Mitcheson and Perry, 2003). Ligand-based in silico models have utilised a range of automated pharmacophore and neural network technologies, with reasonable success (Cavalli et al., 2002; Ekins et al., 2002; Roche et al., 2002; Keseru, 2003; Pearlstein et al., 2003). The promiscuous nature of HERG channel binding (Vandenberg et al., 2001) and the variability in drug potencies complicate the nature of these methodologies; however, these structural and ligand-gated in silico approaches have led to the emergence of the 'drain-plug' picture, wherein inhibitors occlude the channel pore (Pearlstein et al., 2003). Structure–activity relationships for drug targets with a view to reducing HERG channel block are now beginning to emerge (Friesen et al., 2003; Fraley et al., 2004). However, it is unlikely that in silico and in vitro models can fully identify the cardiovascular liability of putative new chemical entities. Indeed, potentially useful compounds which clearly interact with HERG (Fig. 3) but which are not detrimental in vivo (e.g. Verapamil) might be screened out (De Clerck et al., 2002; De Ponti et al., 2002). With reports of attrition rates of up to 10% due to QTc complications (Shah, 2002), this could have serious implications for drug discovery pipelines already under pressure. Fluoxetine may never have been developed (Fermini and Fossa, 2003), as it blocks HERG with an IC_{50} of 1.5 μM in patch clamp studies (Witchel et al., 2002b) and at similar concentrations in the [^{3}H]dofetilide binding assay (K. Finlayson, unpublished observations).

6. Alternative in vitro techniques for assessing torsadogenic potential in man

In light of the previous discussion highlighting the lack of a strong correlation between QT interval prolongation and proarrhythmia (Haverkamp et al., 2000; Hondeghem et al., 2003), alternative and potentially more encompassing high-throughput proarrhythmic models may need to be considered. The SCREENIT system described by Hondeghem et al. (2001, 2003) is an automated computer test apparatus used extensively to characterise drug-induced proarrhythmia in perfused female rabbit hearts. Electrodes are attached to the heart, and the parameters examined include the effects of drug on monophasic action potential duration, intraventricular conduction, instability (index of variation of action potential duration; APD), triangulation (APD_{30} to APD_{90}) and reverse use dependence (Hondeghem and Hoffman, 2003; Hondeghem et al., 2001, 2003). Whether the results in rabbits transfer to other species is unknown (Belardinelli et al., 2003). Like all in vitro studies, there are limitations with the methodology (Hondeghem et al., 2003); however, SCREENIT does appear to be a technique that could be useful as a pre-clinical proarrhythmia test. An alternative approach for examining torsadogenic potential is the arterially perfused canine left ventricular wedge preparation (Antzelevitch and Shimizu, 2002; Di Diego et al., 2003). Using this preparation, the latter authors showed biphasic concentration–response curves for cisapride when inducing QT interval prolongation and TdP. Electrical alterans have recently been identified as an important factor in ventricular fibrillation in connection with the restitution hypothesis (Gilmour, 2003). Using anaesthetised guinea pigs and HERG channel blockers, Fossa et al. (2004) have shown that alterans may indeed be a suitable method with which to discriminate between pro- and non-arrhythmic drugs at therapeutic concentrations. All three methods show that many factors in addition to QT interval prolongation are ultimately required to induce TdP (Belardinelli et al., 2003). Finally, the terms high throughput and zebrafish do not automatically spring to mind when considering HERG. However, Milan et al. (2003) have recently shown that a high percentage of drugs that cause repolarisation abnormalities induced bradycardia and AV block in zebrafish, consistent with effects seen for I_{Kr} blockers in other models (Eckardt et al., 1998; De Clerck et al., 2002). Ultimately, all such methods, of which these by no means form an exhaustive list (see review by Eckardt et al., 1998), will require characterisation and confirmation in other laboratories.

7. In vivo pre-clinical studies

Whole animal models understandably formed an essential part of the pre-clinical battery of studies identified in the original CPMP points to consider document in 1997. At that time, no individual species was identified as being ideal, although it is clear that the dog is the most favoured model (Crumb and Cavero, 1999; Webster et al., 2002; Gralinski, 2003). Species acknowledged as being useful in ICH S7B include dog, monkey, swine, rabbit, ferret and guinea pig. Nevertheless, no model appears to produce TdP with 100% reliability (Eckardt et al., 1998). Studies in dogs, whether conscious or anaesthetised, have their own associated complications; however, they appear invaluable in assessing the arrhythmogenic potential of any new chemical entity

(Eckardt et al., 1998; De Clerck et al., 2002; Gralinski, 2003). Atrio-ventricular block in dogs, a model of enhanced susceptibility for drug-induced TdP, may be useful in this context (Belardinelli et al., 2003). As dog studies are labour-intensive and costly, alternative models such as the anaesthetised guinea pig have been pursued (De Clerck et al., 2002). However, at a molecular level, guinea pigs have a heterogeneity in cardiac ion channel composition, not seen in rabbits, swine and dogs (Crumb and Cavero, 1999; Gralinski, 2003). Methoxamine-challenged rabbits represent a further alternative to dogs for directly assessing the torsadogenic potential of compounds (De Clerck et al., 2002). Without a combination of in vitro and in vivo studies, potentially useful compounds such as sertindole would not exist (Thomsen et al., 2003). Although rodent studies are deemed inappropriate for use in assessing cardiac repolarisation (De Clerck et al., 2002; see ICH S7B), transgenic mouse models with appropriate cardiac ion channel mutations may be helpful in identifying novel arrhythmogenic mechanisms (Tian et al., 2004). Whether any cell-based assay, in vitro heart preparation or even in vivo animal models can predict the potential of drug-induced TdP in humans is the subject of much debate (Belardinelli et al., 2003).

8. Clinical studies

Numerous articles have also discussed the clinical requirements outlined by CPMP in 1997 in relation to QT interval prolongation by non-cardiovascular medicinal products in man (Crumb and Cavero, 1999; Haverkamp et al., 2000; De Ponti et al., 2000, 2002; Malik and Camm, 2001; Fermini and Fossa, 2003). A provisional outline of factors required to be taken into consideration in conducting clinical studies was presented. These included study design, QT interval measurement methodologies, correction formulae and definitions of QTc ranges considered as normal and aberrant. However, whether small clinical trials can ever detect drug-induced changes in QTc interval is debatable (Puddu et al., 2001; Shah, 2002), with some authors even suggesting dynamic QT screening prior to drug prescription. This area is still rapidly evolving and hopefully ICH E14 will help clarify how, and what, clinical studies should be conducted when assessing the arrhythmogenic potential of new chemical entities. Until then, the 'fundamental recommendation is that almost all drugs should have an assessment of its effect on cardiac repolarisation', and that this may ultimately be done by performing a 'thorough QT/QTc study' (see ICH6 presentations on Safety and Efficacy-E14; http://www.ich.org; Morganroth, 2004). Although it may be difficult to establish what is a clinically significant threshold for QT interval prolongation, the naïve reader is given the impression that 'a QTc prolongation of <10 ms (De Ponti et al., 2002), or even 5–10 ms (Haverkamp et al., 2000; Morganroth, 2004) may have serious implications for any new chemical entities. This appears to contrast with the original CPMP document (where changes of <30 ms was considered safe), but it is more in line with recent suggestions by Shah (2002) and Morganroth (2004). With correction formulae inducing a measurement bias of up to 5 ms (De Ponti et al., 2002), large spontaneous changes (Shah, 2002; Morganroth, 2004), and various physiological variations existing as discussed above (3–95 ms), the implications are obvious. Nevertheless, QT interval prolongation, despite its many complications, will undoubtedly continue to be used as a clinical surrogate for TdP.

9. Conclusion

It is only a decade since HERG was originally cloned; however, few would have anticipated the impact that this channel was about to exert. HERG/I_{Kr} and its association with familial and acquired QT interval prolongation and the polymorphic ventricular arrhythmia, Torsades de Pointes, are now fundamental concerns in drug safety studies, whether justifiable or not. With the incidence of Torsades unclear, and the relationship between HERG/I_{Kr}, QT interval prolongation and Torsades ambiguous, only time will tell whether the multitude of studies being utilised/developed will reliably identify a propensity for arrhythmogenecity. As Table 2 clearly shows, a large number of existing medications have been associated with HERG/I_{Kr}, QT interval prolongation and TdP. This is therefore a very complicated area and the necessity for publication of clear pre-clinical and clinical guidelines remains paramount.

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