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REVIEW

In vitro models of proarrhythmia

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Proarrhythmia models use electrophysiological markers to predict the risk of torsade de pointes (TdP) in patients. The set of variables used by each model to predict the torsadogenic propensity of a drug has been reported to correlate with clinical outcome; however, these reports should be interpreted cautiously as no model has been independently assessed. Each model is discussed along with its merits and shortcomings; none, as yet, having shown a predictive value that makes it clearly superior to the others. As predictive as these models may become, extrapolation of results directly to the clinic must be exercised with caution. The use of *in silico* models, from subcellular to whole system, is rapidly beginning to form the first line of screening activity in many drug discovery programmes, indicating that biological experimentation may become secondary to analysis by simulation. *In vitro* proarrhythmia models challenge current perceptions of appropriate surrogates for TdP in man and question existing non-clinical strategies for assessing proarrhythmic risk. The rapid emergence of such models, compounded by the lack of a clear understanding of the key proarrhythmic mechanisms has resulted in a regulatory reluctance to embrace such models. The wider acceptance of proarrhythmia models is likely to occur when there is a clear understanding and agreement on the key proarrhythmia mechanisms. With greater acceptance and ongoing improvements, these models have the potential to unravel the complex mechanisms underlying TdP.
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Keywords: torsades de pointes; proarrhythmia; arrhythmia; action potential duration; QT prolongation; methods; *in vitro*; *in silico* models

Abbreviations: AP, action potential; APD, action potential duration; EADs, early after-depolarizations; hERG, human ether-a-go-go-related gene; I_{Kr}, rapid component of the delayed rectifier potassium current; I_{Ks}, slow component of the delayed rectifier potassium current; M cell, mid-myocardial cell; MAP, monophasic action potential; T, triangulation; TdP, torsades de pointes; TDR, transmural dispersion of repolarization; TRIad, triangulation, reverse-use dependence, instability

Introduction

Most cases of drug-induced torsades de pointes (TdP) have been associated with human ether-a-go-go-related gene (hERG) channel block; however, not all drugs that cause hERG channel block cause TdP (Redfern *et al*., 2003; Milberg *et al*., 2004). Equally, an increase in the QT interval does not *necessarily* lead to TdP. Indeed, there is no straightforward relationship between the degree of drug-induced QT prolongation and the induction of TdP (Yap and Camm, 2003). Thomsen *et al*. (2004) have recently shown that in the presence of D-sotalol, the greatest increase in the QT interval is unaccompanied by a corresponding increase in the incidence of TdP. Thus, it appears that while the standard assays (hERG channel activity, action potential duration (APD) and QT/QTc interval) are very good at predicting the risk of QT interval prolongation in man they are less good at predicting the proarrhythmic risk. As Yap and Camm (2003) have clearly stated, '*…there is no simple relation between the degree of drug-induced QT prolongation and the likelihood of the development of TdP, which can occasionally occur without any substantial prolongation of the QT interval*'. Electrophysiological markers associated with drug-induced TdP other than APD and QT interval prolongation include but are not limited to triangulation (T) of the AP, reverse use-dependence (R), temporal, spatial and transmural dispersion of ventricular repolarization, beat-to-beat variability, the difference in duration between the peak and end of the T wave (T_{peak}–T_{end}, an index of transmural dispersion of repolarization) (Yan and Antzelevitch, 1998), and the incidence of early after-depolarizations (EADs). However, the interplay among these complex electrophysiological events and their respective roles in the genesis and maintenance of TdP is not yet fully understood nor is there agreement on their roles in the maintenance of TdP.

Over the recent years, a number of models have been developed that specifically look at the ability of these electrophysiological events, either singly or in combination,

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to predict TdP in man. Here, we present a summary of three *in vitro* models of proarrhythmia that have significantly advanced our knowledge and understanding of TdP-like arrhythmias and the predictive value of such models to the clinical setting. These methods include (i) the arterially-perfused left ventricular wedge preparation, (ii) SCREENIT, a Langendorff-perfused isolated rabbit heart preparation and (iii) a Langendorff-perfused isolated rabbit heart preparation using bradycardia and hypokalaemia. The rabbit heart has been routinely used in Langendorff preparations primarily because of its sensitivity to TdP-like arrhythmias. This sensitivity results from the rabbit heart having a very low expression of I_{Ks} (slow component of the delayed rectifier potassium current) and therefore having little repolarization reserve (Roden, 1998). A brief description of recent advances towards the development of predictive *in silico* models is also provided.

Arterially-perfused left ventricular wedge preparation

The arterially perfused canine and rabbit left ventricular wedge preparations were originally developed in the laboratory of Charles Antzelevitch (Yan and Antzelevitch, 1998). This model records a pseudo-ECG and three transmembrane APs recorded simultaneously from various layers of the myocardium. Antzelevitch and Shimizu (2002) have identified three distinct regions within the layers of the myocardium that are electrically and pharmacologically distinct: the endocardium, mid-myocardial cell (M cell) region and epicardium. Their intrinsic differences are based on differences in ion channel expression and this manifests as differences in AP configuration and duration, refractory periods and responses to pharmacological intervention (Yan and Antzelevitch, 1998). Simultaneous recordings of APs from each of these cell layers can provide information on the relative differences in APD and repolarization characteristics between the layers and thus provide a measure of dispersion across the wall of the ventricle—a key factor in precipitating arrhythmias (Antzelevitch and Shimizu, 2002). The temporal relationship between these transmural APs and their relationship with the T wave on the ECG is such that repolarization of the epicardial cell AP is coincident with the *peak* of the T wave, and repolarization of the M cell is temporally aligned with the *end* of the T wave. The APD of endocardial cells is usually intermediate. The M cell AP is longer than that of cells in either of the neighbouring regions. This characteristic of M cells is thought to be due to a smaller I_{Ks} current, a larger, sustained sodium current, (I_{Na}), and Na^+/Ca^{2+} exchange current (Antzelevitch and Shimizu, 2002). An interesting finding originating from these studies is that the T_{peak}–T_{end} interval encompasses all the ventricular APDs, clearly illustrating that the T_{peak}–T_{end} interval is a direct measure of transmural dispersion of repolarization (TDR) (Yan and Antzelevitch, 1998). The data show that these differences in time courses of ventricular repolarization appear to be largely responsible for the inscription of the T wave. Pharmacological manipulations that interrupt or slow repolarization of the AP may augment the inherent electrical heterogeneities within the myocardium. For example, on exposure to DL-sotalol, all APs prolong within the ventricle but the M cell AP prolongs more than either the epicardial or endocardial APs, thus widening the T wave (Yan *et al*., 2001). The exaggerated prolongation of the M cell AP causes a greater separation of AP durations between all cell types, with the greatest separation of repolarization times being between epicardial and M cells. Greater separation between the AP durations across the wall of the ventricular myocardium results in an increase in TDR. The DL-sotalol-induced increase in dispersion of repolarization is accompanied by a corresponding increase in the T_{peak}–T_{end} interval and therefore an increase in TDR. In contrast, azimilide, a mixed I_{Kr} (rapid component of the delayed rectifier potassium current) and I_{Ks} blocker, was shown to homogenously prolong epicardial and M cell APDs (at low concentrations, where I_{Kr} block is greater than that by I_{Ks} (Fermini *et al*., 1995) resulting in QT prolongation without an increase in TDR (Yan *et al*., 2001). This was despite the fact that azimilide prolonged the QT interval and M cell APD more than DL-sotalol. Interestingly, at higher concentrations of azimilide ($>3\,\mu M$), where there is substantial block of both I_{Kr} and I_{Ks} (Fermini *et al*., 1995) the change in QT interval no longer followed M cell APD, an effect probably due to preferential prolongation of epicardial APD. The greater increase in epicardial APDs resulted in the generation of EADs from the epicardium (basic cycle length 2000 ms or a stimulation frequency of 0.5 Hz); however, these EADs usually failed to produce R-on-T extrasystoles and TdP (7). This difference in transmural propagation of EADs between DL-sotalol and azimilide was because azimilide decreased TDR, whereas DL-sotalol markedly increased TDR.

An increase in TDR seems to play an important role in determining whether an EAD propagates transmurally to induce an ectopic beat and TdP (Habbab and el-Sherif, 1990). These data point to transmural re-entry as a mechanism for the maintenance of TdP and a transmurally conducted EAD as the initiating mechanism. The findings of Yan *et al*. (2001) suggest that an increase in TDR not only facilitates transmural propagation of EADs but importantly also seems to contribute to the maintenance of TdP. This is consistent with the clinical observation that patients with congenital long QT (LQT) syndrome have longer T_{end}–T_{peak} intervals and therefore an increased TDR (Lubinski *et al*., 1998).

The pivotal finding from the left ventricular wedge preparation is that augmentation of the ventricular myocardium's natural heterogeneity by an increase in TDR is a consequence of the preferential prolongation of the AP in the M cell, leading to TdP. Yan *et al*. (2001) suggest that TDR is better correlated with human TdP than the length of the QT interval; however, further independent validation is required to substantiate this claim.

SCREENIT: an automated Langendorff-perfused isolated rabbit heart preparation

The SCREENIT Langendorff-perfused isolated rabbit heart preparation was developed by Luc Hondeghem. SCREENIT is a fully automated computerized screening model designed to

identify pro-, anti- and non-arrhythmic compounds with moderate throughput (Hondeghem *et al*., 2001). The basic premise behind this model is that AP prolongation is not the only factor that predisposes the heart to arrhythmogenesis. Hondeghem has proposed that the shape of the AP and the stability of the prolongation are more critical than prolongation *per se*. The TRIad is a term coined by Hondeghem to describe these changes and represents triangulation (T), reverse use-dependence (R) and instability (I) (beat-to-beat variations in monophasic APD). In addition to prolongation, these characteristics may be used as surrogate markers of proarrhythmia in man (Hondeghem *et al*., 2001). In this model, monophasic AP (MAP)-recording electrodes are placed on epicardial and endocardial ventricular surfaces to record TRIad elements, $APD_{10–90}$, conduction velocity and proarrhythmic events such as EADs.

It has been suggested that not all AP prolongations are proarrhythmic (Hondeghem *et al*., 2001; Milberg *et al*., 2004). Prolongation of the AP in the plateau region, phase 2, rather than phase 3 of AP repolarization produces a 'square' AP. This sort of prolongation is antiarrhythmic because rapid repolarization and a positive plateau potential are maintained. Prolongation that is antiarrhythmic can be represented on the Poincaré plot (derived by plotting the duration of one AP against the duration of the next AP, and so on) as travel along the diagonal line but in the absence of deviations from the identity line, indicating that there is little beat-to-beat variability in APD and that the preparation is very stable. AP prolongation that is proarrhythmic produces a triangulated AP (slowing phase 3 of AP repolarization thereby increasing the time spent in the Na^+ and Ca^{2+} activation windows) causing an extreme increase in APD at long (2000 ms) cycle lengths, indicating reverse use-dependency and displays complex polygons around the diagonal line of the Poincaré graph indicative of large beat-to-beat variability in APD and a very unstable preparation (Hondeghem *et al*., 2001).

The SCREENIT model and the TRIad concept have been tested in three validations with the aim to determine whether the model could discriminate between pro-, anti- and non-arrhythmic drugs and therefore to determine the predictive value of this model to TdP in man. These validation studies have included a number of vehicles and a wide range of marketed drugs from a range of therapeutic indications. Three of these validations have already been published (Hondeghem and Hoffman, 2003; Hondeghem *et al*., 2003; Lawrence *et al*., 2006). The main findings arising from these studies are (i) in general, using SCREENIT and TRIad analysis, proarrhythmic drugs were correctly categorized, (ii) TRIad elements precede overt proarrhythmia and (iii) the TRIad elements probably act synergistically (Hondeghem *et al*., 2001) so that as single occurrences they are less dangerous, whereas collectively they herald the onset of an acute arrhythmogenic substrate. These validation studies were developed to determine whether TRIad elements occur, and if so, which ones; whether there was a rank order of TRIad elements; whether they can be used as surrogate markers of proarrhythmia and if they can predict the clinical situation. However, real value would be added if effects or signals could be expressed relative to free plasma concentration (unbound) and/or the total (free + bound) drug concentration found in the heart (Redfern *et al*., 2003; Titier *et al*., 2004).

To summarize, triangulation, reverse use-dependence and instability describe characteristic changes of the AP on exposure to some drugs. These elements are collectively referred to as the TRIad and can be used as surrogate markers of proarrhythmia. The SCREENIT model and TRIad analysis have undergone extensive (blinded) pharmacological validation, discriminating pro- from anti- and non-arrhythmic drugs (Hondeghem and Hoffman, 2003; Hondeghem *et al*., 2003; Valentin *et al*., 2004; Lawrence *et al*., 2005). However, these results need to be reproduced in multiple laboratories before SCREENIT can be fully accepted as a validated model.

Manual Langendorff-perfused isolated rabbit heart preparation

Another variant of the Langendorff-perfused isolated rabbit heart preparation is a model originally developed in the 1990s (Franz *et al*., 1992; Zabel *et al*., 1997). More recently, Haverkamp and Eckardt have further developed this model to explore the characteristics of class III drug-induced ventricular arrhythmias (Eckardt *et al*., 1998; Johna *et al*., 1998). In contrast to the SCREENIT model, bradycardia, achieved by chronic atrioventricular block, and hypokalaemia are necessary interventions making it more sensitive to proarrhythmic agents. Distinguishing features of this model include simultaneous recordings from eight MAP suction electrodes, a 12-lead ECG and left ventricular developed pressure. The multiple MAP recordings and the specific placement of the electrodes allow not only measurements of triangulation but also of interventricular and transmural dispersion, and regional differences between apex and base.

Milberg *et al*. (2004) have shown that DL-sotalol (100 μM) increases dispersion of repolarization with increasing cycle length, whereas amiodarone had no significant effect on dispersion at any cycle length compared with control. EADs and triggered activity were seen in 50% of hearts treated with DL-sotalol, and all episodes of TdP were associated with EADs. In contrast, no EADs or TdP were seen in the presence of amiodarone in spite of significant increases in the QT interval. These experiments show that the AP-prolonging effect of DL-sotalol occurred mainly during phase 3 repolarization, thus leading to a triangulated AP and creating a proarrhythmic substrate. However, amiodarone prolonged the AP (by a comparable duration), in phase 2 of the AP, resulting in a 'squaring' of the AP. The AP was prolonged and therefore delayed; however, repolarization was better synchronized. Although both drugs induce significant and comparable APD (and QT interval) prolongation, the results show that they have quite different potentials to induce EADs and TdP. Triangulation of the AP, in turn, is associated with increased time in the activating voltage range for Na^+ and Ca^{2+} channels. This is the vulnerable period in which these channels may recover from inactivation and then conduct a depolarizing current, thus generating an EAD. The majority of drugs that induce EADs act via at least one of the following four ionic mechanisms: (i) inhibition of

repolarizing K^+ currents, (ii) increase in the L-type Ca^{2+} current, (iii) increase in the late or persistent Na^+ current or (iv) any combination of these.

Recently, Milberg *et al*. (2005) have developed a novel model of LQT3. They have shown that an inhibitor of sodium channel inactivation reproducibly mimics LQT3 and that TDR plays a major role in the genesis of EADs and TdP in this model.

In summary, Milberg *et al*. (2005) have concluded that the causes of TdP include dispersion of repolarization, triangulation and reverse use-dependence. In addition, it was highlighted that antiarrhythmic properties of drugs are likely to include a rectangular or square configuration of the AP in the presence of prolongation, and rapid phase 3 repolarization, such that AP repolarization may be delayed but not slowed.

Table 1 makes comparisons among the *in vitro* proarrhythmia models discussed above. Particular features of the models are compared, including throughput, technical challenge and proarrhythmic indices measured. Other issues such as the extent to which the model has been validated and its predictive value to man are also considered. Clearly the automation inherent in the SCREENIT model allows a much higher throughput and, in turn, a greater number of drugs have been tested to validate this model. A consequence of the greater degree of technical skill required to perform the wedge preparation technique relative to other models may extrapolate to a lower success rate for the inexperienced technician. However, the data provided by the pseudo-ECG makes this a worthwhile endeavour. An advantage of the manual Langendorff model is that it is a simpler experimental set-up than the other models, and therefore the required technical expertise does not have to be as great to maintain a high experimental success rate. The multiple ECG and MAP recordings mean that it is not critical for all electrodes to remain in place for the duration of the experiment. Fewer marketed drugs have been through the manual Langendorff model; however, its simplicity and high-quality data make it an attractive option. Because fewer drugs have been tested in this model, it has been given a slightly reduced score for 'predictive value to man' than the wedge preparation and the SCREENIT model, this primarily reflects the novelty of the model, not necessarily its predictive value.

An exciting and perhaps technically easier and data-rich alternative to microelectrode recordings from the wedge preparation is the use of optical imaging (Akar *et al*., 2002). The optical mapping system is capable of measuring electrical heterogeneities across the ventricular wall and provides a quantitative assessment of TDR under various electrophysiological conditions. Optical mapping experiments have shown that large repolarization gradients are of greater importance for predicting TdP than the change in magnitude of APDs (Liu and Laurita, 2005). Technical advances suggest that optical mapping will continue to make great contributions to cardiac electrophysiology.

Finally, advances in computer modelling illustrate that the role of *in silico* biology is becoming increasingly prominent as we seek to exploit the rapid growth in biological databases. The use of *in silico* models, from subcellular to whole system, is rapidly beginning to form the first line of

Table 1 Comparisons among *in vitro* proarrhythmia models

Parameters	*Langendorff heart*		
	Wedge preparation	*Manual method*	*Automated SCREENIT model*
Throughput	− (low)	+	+ +
Success rate (%)	>50	>80	>80
Technical challenge	Very high	High	Low-medium
Possibility of inter-laboratory use	Yes	Yes	No
Proarrhythmic indices	TAPs, ECG, APD_{90}, TDR, QT interval T_{peak}–T_{end}, EADs, TdP	MAPs, ECG, LVP, T, R, $APD_{10–90}$, transmural and spatial dispersion, QT interval, EADs, TdP	MAPs, CV, coronary perfusion, T, R, I, $APD_{10–90}$, EADs,
Pharmacological validation	>20 drugs on market	~10 drugs on market	>100 drugs on market
Predictive value in humans	+ +	+ +	+ + +

Abbreviations: APD, action potential duration at 90% repolarization; EAD, early after-depolarization; I, instability; LVP, left ventricular pressure; MAP, monophasic action potential; R, reverse-use dependence; T, triangulation; TAP, transmural action potential; TDR, transmural dispersion of repolarization; TdP, torsade de pointes. Three *in vitro* proarrhythmia models: (i) the wedge preparation, (ii) the manual Langendorff-perfused heart model: SCREENIT and (iii) the automated Langendorff-perfused heart model. 'Throughput' is based on the number of compounds that can be tested per day. The automation inherent in the SCREENIT model allows a much higher throughput. 'Preparation viability' is the robustness of preparation. The Langendorff-perfused heart has been extensively used for over 100 years, its prolonged existence being due to the superior stability and longevity of the preparation. The 'technical challenge' required to maintain multiple intracellular recordings from a wedge preparation is more technically challenging than a Langendorff-perfused heart. The manual Langendorff-perfused model has been considered the simplest model, technically, as (i) the placement of external stimulating electrodes does not rely on the heart's intrinsic electrical circuitry, as does the SCREENIT model, and (ii) monophasic action potential electrodes can be placed and adjusted manually as required. At present there is no opportunity to make inter-laboratory comparisons with the SCREENIT model; however, both the wedge preparation and the manual Langendorff-perfused heart model are used in other laboratories. The parameters measured by each model are listed. The wedge preparation and the manual Langendorff-perfused heart model record an ECG as well as action potential parameters. The multiple ECG and MAP recordings from the manual Langendorff-perfused heart model mean that it is not critical for all recording electrodes to remain in place for the duration of the experiment. The size of the validation test sets is listed. These validations help to determine the predictive value of the model to findings in the clinical setting and hence provide confidence in the ability of the model to reflect and predict clinical outcomes. The evidence for the predictive value of each model is based on the findings from the validation test sets and published clinical findings. The slightly lower rating of the manual Langendorff-perfused heart model reflects the significantly fewer marketed drugs that have been tested in this model. In this case, the reduced score primarily reflects the novelty of the model, not necessarily its predictive value.

screening activity in many drug discovery programmes, clearly indicating that biological experimentation may become secondary to analysis by simulation.

In silico models

Computational models for profiling proarrhythmic risk have made significant advances in recent years. Highly sophisticated *in silico* models have been developed to predict hERG channel activity (Cavelli *et al*., 2002), AP duration in rabbit, canine and human ventricular myocytes and/or Purkinje fibres (Luo and Rudy, 1994; Ten Tusscher *et al*., 2004) and electrical wave propagation throughout the intact myocardium (Winslow *et al*., 1999; Crampin *et al*., 2004; Noble, 2004).

Cavelli *et al*. (2002) have designed a pharmacophore for LQTS-inducing drugs together with a 3D quantitative structure–activity relationship (QSAR) model for hERG channel-blocking activity. The former was derived from a list of known QT-prolonging compounds. To further develop this model as a predictive virtual screening tool, a sufficiently large database of reliable and homogenous data is required. Highly potent drugs such as astemizole bind in the sterically favourable regions C1 and C2, whereas less potent drugs such as gatifloxacin bind in the space around C0, which seems sensitive to both steric and electrostatic properties.

Hund and Rudy (2004) have developed a detailed and physiologically based mathematical canine ventricular cell model that may serve as a valuable tool in early drug development. This model is similar to the Luo and Rudy (1994) model; however, it incorporates dynamic Ca^{2+}/calmodulin activity and regulation of intracellular Ca^{2+} handling, the late Na^{+} current and the Ca^{2+}-dependent I_{to} (transient outward current). These modifications set it apart from previous models. Computational models of AP prolongation are potentially powerful tools for investigating normal and abnormal myocardial behaviour in research and drug discovery.

In silico models of the whole heart can simulate normal sinus rhythm, with the wave of excitation spreading rapidly throughout the ventricles. The resulting virtual ECG closely resembles normal rhythm (Winslow *et al*., 1999; Crampin *et al*., 2004). In contrast, when an area of the heart is reprogrammed to reproduce the selective downregulation of K^{+} channels characteristic of heart failure, an arrhythmia can be induced *in silico*. The initiating event is an EAD from a K^{+} channel-deficient zone. Once triggered the arrhythmia is perpetuated by rapid waves of depolarization spreading asynchronously throughout the heart. Virtual ECGs reveal the undulating waveform of polymorphic ventricular tachycardia. Given the complexity of cardiac arrhythmias, such *in silico* simulations will undoubtedly feature more prominently in future investigations.

The goal of cardiac modelling is a fully integrated electromechanical model of the human heart that describes normal and pathological processes at the molecular, cellular, tissue and organ levels simultaneously. However, vast amounts of experimental data are required to train *in silico* models to increase their predictive value: they will only ever be as good as our current knowledge, so although they are important tools in drug discovery, they have less relevance in investigating underlying disease mechanisms. The availability of *in silico* methods in the early phase of drug development would dramatically increase the screening rate and would also lower the costs compared with experimental assays. In the future, *in silico* models are likely to become more routinely used to predict normal and pathophysiological processes, and thus they are poised to revolutionize biomedical research and drug discovery.

There is no single, standardized and uniform method for non-clinical screening of the proarrhythmic effects of drugs on the heart. *In vitro* proarrhythmia methods lack the ability to provide data on pharmacodynamic processes and suffer from having restricted functional end points outside the confines of arrhythmias. However, these methods have the advantage that the ionic and pharmacologic milieu and pacing frequency can be easily manipulated, and the assay is not restricted by dose-limiting toxicity. Importantly, it is possible to employ *in vitro* methods early in the drug discovery process so that compound attrition at later stages may be reduced, therefore, ensuring progression of safe drugs. It must be acknowledged that among the various methods available none has demonstrated a predictive value that makes it clearly superior to the others.

Conclusion

Multiple mechanisms are increasingly recognized as contributing to normal repolarization. Roden (1998) introduced the concept of repolarization reserve to explain congenital variability in response to reduction or block of I_{Kr}. Reduced I_{Kr} may not lead to clinical consequences, if, as proposed, I_{Ks} remains intact and functions as a major source of 'repolarization reserve' that protects against TdP during I_{Kr} block. It is clear that none of the 'gold standard' QT-related assays (hERG, APD and *in vivo* QT assays) alone can sufficiently predict the risk of TdP in man nor is there consensus on the parameters that best predict proarrhythmia in man, highlighting the complexity of the issue and the need for proarrhythmia models that encompass most if not all of the predisposing factors. The emergence of proarrhythmia models and in particular *in vitro* and *in silico* models is a testament to the prevalence of the problem and the urgency with which it is being tackled. Although there may be disagreement on which is the most predictive proarrhythmia model or variable(s), of greater importance is identifying the central themes derived from these models. The foremost key component linked to the induction of TdP is the development of EADs. Without exception, EADs were identified in every model as being primary to the induction of TdP. Of equal importance is identifying the components critical to the development of EADs. Emerging as primary components leading to EADs are triangulation of the AP and dispersion of repolarization, specifically temporal (instability of the APD also called alternans) and transmural dispersion.

Although proarrhythmia models show promise in being able to form a bridge between QT prolongation and

proarrhythmia, as well as between non-clinical and clinical data, there is understandable regulatory reluctance to accept the value of these models, especially where there appears to be interlaboratory variability. Acceptance of these models may prove to be challenging given the implications of the clinical guidance document E14 in which non-clinical data are given little emphasis (Anonymous, 2005a, b). Perhaps surprisingly, still much emphasis is placed on the absence of QT interval prolongation in phase 1 clinical trials (Franz *et al*., 1992; Hondeghem *et al*., 2001; Antzelevitch and Shimizu, 2002; Milberg *et al*., 2002, 2004; Belardinelli *et al*., 2003; Yap and Camm, 2003; Poelzing and Rosenbaum, 2004; Thomsen *et al*., 2004). The discrepancy between QT interval prolongation and proarrhythmia is a significant concern for clinicians, pharmaceutical companies and regulatory authorities.

In summary, the integration of data generated from a proarrhythmia model with data generated from QT-related assays would allow better predictions for assessing the risk of TdP in man and therefore aid decision-making efforts for the selection and development of safer drugs.

Conflict of interest

The authors state no conflict of interest.

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