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RESEARCH PAPER

A Rabbit Langendorff Heart Proarrhythmia Model: Predictive Value for Clinical Identification of Torsades de Pointes

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Background and purpose: The rabbit isolated Langendorff heart model (SCREENIT) was used to investigate the proarrhythmic potential of a range of marketed drugs or drugs intended for market. These data were used to validate the SCREENIT model against clinical outcomes.
Experimental approach: Fifty-five drugs, 3 replicates and 2 controls were tested in a blinded manner. Proarrhythmia variables included a 10% change in $MAPD_{60}$, triangulation, instability and reverse frequency-dependence of the MAP. Early after-depolarisations, ventricular tachycardia, TdP and ventricular fibrillation were noted. Data are reported at nominal concentrations relative to $EFTPC_{max}$. Proarrhythmic scores were assigned to each drug and each drug category.
Key results: Category 1 and 2 drugs have the highest number of proarrhythmia variables and overt proarrhythmia while Category 5 drugs have the lowest, at every margin. At 30-fold the $EFTPC_{max}$, the mean proarrhythmic scores are: Category 1, 101 ± 24; Category 2, 101 ± 14; Category 3, 72 ± 20; Category 4, 59 ± 16 and Category 5, 22 ± 9 points. Only drugs in Category 5 have mean proarrhythmic scores, below 30-fold, that remain within the Safety Zone.
Conclusions and Implications: A 30-fold margin between effects and $EFTPC_{max}$ is sufficiently stringent to provide confidence to proceed with a new chemical entity, without incurring the risk of eliminating potentially beneficial drugs. The model is particularly useful where compounds have small margins between the hERG IC_{50} and predicted $EFTPC_{max}$. These data suggest this is a robust and reliable assay that can add value to an integrated QT/TdP risk assessment.
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Keywords: arrhythmia; cardiac; early after-depolarisations; electrophysiology; *in vitro*; map; monophasic action potential; proarrhythmia; QT prolongation; Torsades de Pointes

Abbreviations: CYP3A4, cytochrome *P*450 enzyme isoform 3A4; EADs, early after-depolarisations; $EFTPC_{max}$, maximum effective free therapeutic plasma concentration; hERG, human ether-a-go-go-related gene; I, instability; I_{K1}, inward rectifier potassium current; I_{KACh}, acetylcholine-activated potassium current; I_{KATP}, adenosine triphosphate (ATP)-activated potassium current; I_{Kr}, rapid component of the delayed rectifier potassium current; I_{Na}, sodium current; I_{to}, transient outward current; MAP, monophasic action potential; MAPD, monophasic action potential duration; $MAPD_{60}$, monophasic action potential duration at 60% repolarisation; PDE-5, phosphodiesterase-5 inhibitor; QTc, corrected QT interval; R, reverse frequency-dependence; T, triangulation; TdP, Torsades de Pointes; TRIad, triangulation, reverse frequency-dependence and instability; VF, ventricular fibrillation; VT, ventricular tachycardia

Introduction

The duration of the QT interval on the electrocardiogram (ECG) is used as a clinical surrogate marker to determine the risk of a particular drug-induced arrhythmia: Torsades de Pointes (TdP). However, while most cases of proarrhythmia are preceded by QT prolongation, proarrhythmia can sometimes occur without any appreciable QT prolongation and equally, the QT interval may be greatly prolonged without any signs of proarrhythmia (Antzelevitch and Shimizu, 2002; Milberg *et al.*, 2002; Belardinelli *et al.*, 2003; Yap and Camm, 2003). While being an inconsistent and unreliable measure of drug-induced proarrhythmia, QT prolongation, as judged by regulatory authorities, is currently the only clinical variable used as a possible predictor of drug-induced proarrhythmia. However, QT interval prolongation is a crude indicator of torsadogenic risk in both humans and animals.

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It is clearly preferable to discover that a compound is proarrhythmic before clinical development or marketing strategies are initiated. Non-clinical testing strategies often aim to detect the risk of QT prolongation in man, but not necessarily the proarrhythmic risk (Anon, 2005a, b). Consequently, more sophisticated tools and analysis are required to refine non-clinical and clinical predictors of torsadogenic risk. Recently, models of proarrhythmia have been used as part of non-clinical integrated cardiovascular risk assessments (Lawrence *et al*., 2005). While there is currently no consensus on the parameters that best predict proarrhythmia in man, the collective purpose of these models is to: (i) determine better markers of proarrhythmic risk; (ii) be able to predict the propensity of a drug to induce TdP; (iii) aid in the discovery and development of safer drugs; and (iv) minimise the risk that a safe and clinically relevant drug will be erroneously rejected.

In vitro proarrhythmia models are being used with greater regularity in the drug discovery process, and this may lead to their gaining wider acceptance within the scientific and regulatory community, particularly if the strengths and limitations of the models are readily acknowledged. In this study, we have focussed on a single proarrhythmia model: the rabbit isolated Langendorff heart model (SCREENIT). The primary objective of this investigation was to determine if the model could accurately predict the proarrhythmic risk to humans of a range of marketed drugs or drugs intended for market. In particular, we set out to: (i) determine the reproducibility of the experimental method; (ii) identify the frequency of false positives and false negatives and (iii) define the concordance between the non-clinical results, therapeutic concentration and clinical outcome. We provide an independent assessment of the proarrhythmia model SCREENIT. In addition, the magnitude and range of this study (55 marketed drugs or drugs intended for market for a range of therapy areas, plus three replicates and two controls tested in duplicate) allows for thorough assessment and robust conclusions of drug effects in this model.

Methods

Experiments were performed at Hondeghem Pharmaceutical Consulting, Oostende, Belgium. All experimental protocols conformed to the Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 852-3, revised 1996).

Experimental preparation

The method has been described previously in detail (Hondeghem, 1994; Hondeghem *et al*., 2001; Lawrence *et al*., 2005). Briefly, 2.4–2.6 kg female New Zealand white rabbits (Hondeghem *et al*., 2001) ($n=312$) were stunned by a captive bolt. After midsternal incision and opening of the pericardium, hearts were removed and immediately placed in cold Krebs–Henseleit buffer (composition in mM: NaCl 118, KCl 4, $NaHCO_3$ 22, $MgCl_2$ 1.1, NaH_2PO_4 0.4, $CaCl_2$ 1.8, dextrose 5, pyruvate 2 and creatine 0.038). The aorta was cannulated, the bundle of His was sectioned and stimulating electrodes were sutured to each side of the distal His bundle. Hearts were perfused at a constant pressure of 80 cm H_2O with Krebs–Henseleit solution warmed to 33–35°C. The perfusate was equilibrated with 95% O_2 and ~5% CO_2 to maintain the pH at 7.35. A monophasic action potential (MAP) recording electrode was advanced into the left ventricle until it reached the subendoardium, a region rich in Purkinje fibres. A second MAP recording electrode and a reference electrode were positioned on the left ventricular epicardium. The reference electrode was perfused at about 1 ml/min with isotonic KCl, enriched with 1.8 mM $CaCl_2$, and grounded. Once the heart was mounted on the experimental station, the execution and analysis of the experiment proceeded without further human intervention. Hearts were stimulated at 1.5 times threshold stimulation. If automaticity and escape cycle length were >1000 ms, threshold stimulation current $<300\,\mu$A, coronary perfusion >17 ml/min, ectopic rate <40 ectopics for a 10 min interval, the cardiac activation time <60ms, and the product of the number of ectopic beats $\times$ instability ((beats/min)ms) <750 ms, then the preparation was stimulated until instability of MAP duration (MAPD) (determined by the Best Easy Systematic method (BES) as defined by Wonnacott and Wonnacott (1977) and described below) of the last 20 consecutive MAP trains became <10 ms. Preparations that did not achieve these criteria (approximately 10%) were rejected. The data were compressed and stored on removable disks.

Experimental protocol

The experiments carried out for this study were conducted between 2002 and 2005. During this time, slight modifications may have been made to the computer algorithm that defines the experimental methodology. As a result, the experimental method may not be identical for all drugs; however, it is believed that the methodologies are sufficiently similar to consider all experiments together.

The experiment consisted of small protocols executed every minute at every concentration and a large protocol that was executed at the end of the control period (10–15 min) and at the highest drug concentration (Figure 1). In the small protocol, MAPs of a 10-beat train at 1000 and 300 ms were recorded together with a train of 30 MAPs stimulated at a cycle length of 1000 ms. On odd numbered minutes a 30 s MAP train with irregular stimulation intervals simulated long/short/long sequences as follows: five cycles at 1 s, followed by an irregular sequence: 0.4, 1.8, 1, 0.75, 0.45, 0.6, 2, 0.45, 0.45, 2.6, 2, 0.5, 0.9, 0.9, 1.2, 0.3, 0.3, 0.3, 1.1 and 2 s and terminated by five more cycles at 1 s. In the large protocol, automaticity, escape cycle lengths, conduction times and MAPDs at cycle lengths of 2000, 1500, 1000, 750, 500, 300 and 250 ms were determined. The large protocol was about 20 min in duration.

In a typical experiment, the preparation was perfused with drug-free solution while the small protocol was executed 10 times, followed by the large protocol (control data). Drug infusion at the lowest drug concentration was then started and the small protocol was executed for 10–15 min. This was

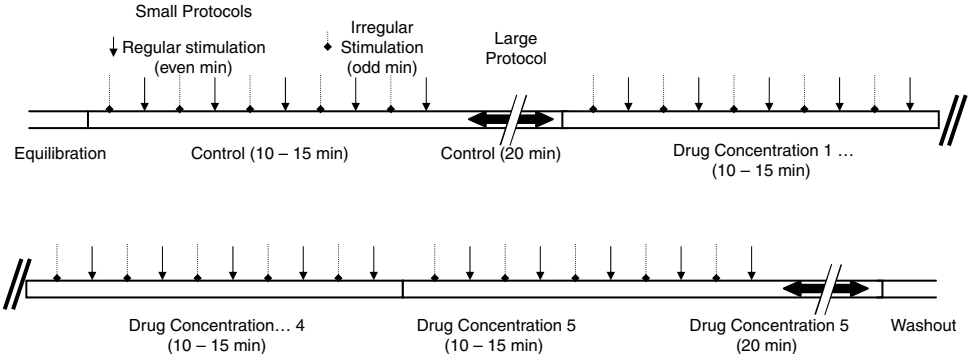

Figure 1 Experimental protocol. The experimental protocol consisted of small and large protocols initiated after an equilibration period. Every first, third, fifth etc minute irregular stimulation frequencies were executed to simulate long/short/long sequences. The small protocol was executed 10 times and was 10–15 min in duration. In control solution and at the highest test concentration the large protocol was executed.

continued for all subsequent drug concentrations: 2nd, 3rd, 4th and 5th. At the 5th and highest drug concentration, the small protocol was followed by the large protocol, which was executed for about 20 min, and finally the heart was perfused with drug-free solution as a washout (Figure 1).

Electrophysiological measurements

Electrophysiological measurements were made using the SCREENIT system (Hondeghem, 1994). MAPs were digitised at 1 kHz (12-bit resolution) and saved to disk. The MAP upstroke was sampled at 10 kHz. Amplitudes of MAPs were required to be >4 mV and exceeding $>50\%$ of the average upstroke in a MAP train to be considered valid. $MAPD_{10–90}$ were measured from the midpoint of the upstroke until 10, 20,…,90% repolarisation. A 10% increase in the average $MAPD_{60}$ was considered a significant prolongation (Gintant *et al*., 2001). Triangulation was measured as the repolarisation time from $MAPD_{30}$ to $MAPD_{90}$. Triangulation was considered significant if the change in repolarisation time was >29 ms (Hondeghem *et al*., 2001). Reverse frequency-dependence was measured as the difference between the $MAPD_{60}$ of the first 10 and that of the last 20 MAPs of a 30-pulse train at 1 Hz (Hondeghem *et al*., 2001). A difference >6 ms as compared to that in drug-free solution was considered as significant frequency-dependence. An instability value >14 ms was considered significant (a detailed description is given below in Data acquisition and statistical analysis). Significance levels (in ms or as a percentage) were determined by analysis of 152 control experiments (Hondeghem *et al*., 2003). Any MAPs where the upstroke was not within 80 ms after the stimulus was considered an ectopic beat. Repolarisation disturbances recorded from a MAP electrode result from ectopic beats or differences in cellular repolarisation times at local or remote sites from the MAP electrode. The number of ectopics was reported as an average (ectopics/10 min) during the last 3 min of any 10–15 min perfusion (Hondeghem *et al*., 2001). For simplicity, the severity of these ectopics has been classed as follows (Figure 2). A positive voltage deflection interrupting late phase 2 or phase 3 of MAP repolarisation was regarded as an early after-depolarisation (EAD). These should not be confused with 'true' EADs, however, that are recorded from a transmembrane recording electrode. Ventricular tachycardia (VT) was defined as >6 consecutive beats with similar appearance. TdP was identified when >3 beats with positive and negative deflections appeared on the MAP, and where the MAP amplitude and upstroke were variable, but oscil-

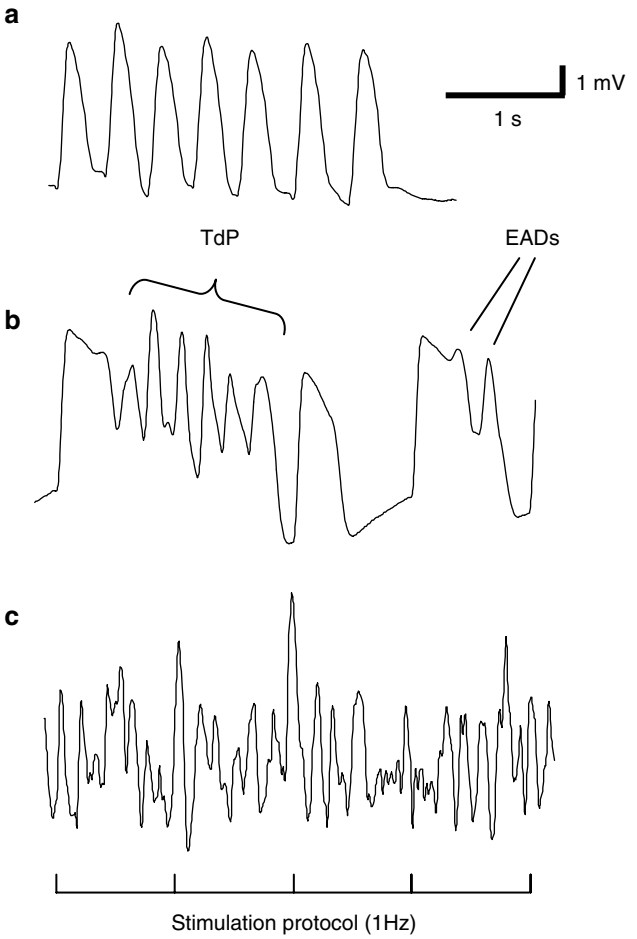

Figure 2 Repolarisation disturbances recorded from an MAP electrode arise from ectopic beats or differences in cellular repolarisation time. The severity of ectopics is classed as (**a**) VT, (**b**) EADs and Torsades de Pointes and (**c**) VF. The stimulation protocol is shown below the traces.

lated with a predictable pattern, similar to that seen as prototypical twisting of the ECG around the isoelectric line. Ventricular fibrillation (VF) was defined when consecutive beats appeared random with no reproducible pattern. If proarrhythmic activity became too great the computer automatically terminated the experiment. These experiments were nevertheless included in the analysis up to the point of excessive proarrhythmic activity and automatic termination, and the particular proarrhythmia was noted.

Data acquisition and statistical analysis

Data were captured by a 12-bit analogue to digital converter sampling each of the two channels: subendocardial and epicardial MAPs. Data were analysed beat by beat during the experiment, compressed and saved to disk. Following the experiment, a detailed analysis of the data was executed. The data were stored in an ASCII format.

Instability of the $MAPD_{60}$ was assessed with a non-parametric test because it is not possible to assume that $MAPD_{60}$ is distributed normally during drug perfusion. To minimise the bias introduced by a few exceptionally long or short MAPDs, the BES method was used to estimate the $MAPD_{60}$. This entails sorting steady-state MAPs according to their $MAPD_{60}$, and by linear interpolation, the median, upper 25% and lower 25% values were computed. An instability value was obtained by computing the difference between the upper and lower quartile estimates in milliseconds. For the experimental MAP trains in each drug concentration, the last 20 MAPs for the final 3 min of drug perfusion in the short protocol were used, a total of 60 MAPs. A difference between control and drug effects >14 ms was considered significant.

Drugs were categorised according to the classification outlined by Redfern *et al*. (2003). Category 1 included repolarisation prolonging (Class Ia and Class III) antiarrhythmics (which have I_{Kr} block as an integral pharmacodynamic mechanism and QT prolongation as an intended desirable effect) with frequent TdP reports; Category 2 included drugs that were withdrawn or suspended from the market due to an unacceptable risk of TdP (although the frequency may have been very low); Category 3 included drugs that have a measurable incidence of TdP in humans or for which numerous case reports exist; Category 4 included drugs for which there have been isolated reports of TdP in humans; and Category 5 included drugs for which there have been no published reports of TdP in humans.

In most cases six animals were used per drug ($n=6$), however, because experiments may be terminated automatically according to criteria defined previously (Hondeghem *et al*., 2001), experiment numbers may vary depending on concentration and drug.

Drugs

All drugs and controls were supplied in sequentially numbered vials (1–62) by AstraZeneca and sent to Hondeghem Pharmaceutical Consulting. Drugs were either synthesised by and/or on behalf of AstraZeneca, Alderley Park or sourced from either Sigma Aldrich or Apin Chemicals. Some drugs were tested in duplicate. In this blinded study, molecular weights were not provided. The highest test concentration was obtained by adding a defined volume of DMSO (not exceeding a final concentration of 0.1%) directly to the vial of drug supplied. Wherever possible, drugs were tested at five concentrations spaced at either full- or half-log intervals previously determined as 1 (occasionally <1), 10-, 30-, 100- and 300-fold the highest effective free therapeutic plasma concentration ($EFTPC_{max}$) in man (Redfern *et al*., 2003; DRUGDEX®, 2005). All drug concentrations are nominal since drug concentrations in the perfusate were not analysed.

Results

Proarrhythmic score

The main challenge in analysing data from this study has been to condense several proarrhythmia variables into a meaningful, ideally single, numerical value or proarrhythmic score. It has been shown that a scoring system is a valid and sensitive approach to facilitate the quantitative analysis of (pro)arrhythmias or for model building (Curtis and Walker, 1988). However, it is emphasised that (pro)arrhythmia scores should be considered in relation to concentration–response data and in conjunction with the raw (pro)arrhythmia data (Curtis and Walker, 1988). Although we cannot define whether or not the data are distributed normally we have used the standard error (s.e.) of the mean as an indication of the spread/variability of the data across concentrations and drug categories. We have made a preliminary attempt to combine all signals of proarrhythmia into a single score to assess proarrhythmic risk, at the highest EFTPC and at multiples above the highest EFTPC (concentration–response data). The proarrhythmic potential of drugs was determined by assigning a weighted score to proarrhythmia variables: overt proarrhythmia (EADs, VT, TdP and VF), $MAPD_{60}$ prolongation, triangulation, reverse frequency-dependence and instability. Weightings were assigned from this data set based on the sensitivity of each variable ($MAPD_{60}$ prolongation, triangulation, reverse frequency-dependence and instability) to the incidence of overt proarrhythmia (EADs, VT, TdP and VF) for a given drug. Covariate analysis (Spotfire software) showed that instability of MAPD was highly sensitive to the incidence of proarrhythmia while reverse frequency-dependence was the least sensitive to the incidence of overt proarrhythmia. Instability of MAPD was the most frequent *first* proarrhythmic variable overall, as well as in most of the drug categories. Triangulation was considered to pose a greater proarrhythmic risk than reverse frequency-dependence because it causes additional vulnerability to EADs due to slowing of repolarisation and therefore an increase in the duration spent in the voltage range for Na^+ and Ca^{2+} channel (re)activation. Drug-induced MAPD prolongation is greater at lower heart rates (reverse frequency-dependence) and this effect increases the risk of EAD development, but without the additional impact of slow repolarisation. Prolongation of the MAPD has both anti- and pro-arrhythmic properties: while being an antiarrhythmic mechanism for Class III

antiarrhythmic drugs, significant prolongation, particularly if combined with repolarisation slowing, may also render the heart susceptible to EADs and proarrhythmia. Based on these data, scores have been weighted as follows: instability 20 points, triangulation 10 points, reverse frequency-dependence 5 points. The sum of these values is averaged according to the number of experiments for each drug at each concentration. Ten points are then added to this value if there is a greater than 10% increase in the average $MAPD_{60}$, and a further 100 points are added for the first incidence of overt proarrhythmia, for example, EADs, VT, TdP and VF (successive occurrences of overt proarrhythmia are not included). Scores are cumulative so that points from previous concentrations are added to the sum of successive concentrations. The cumulative score may be misleading, however, since some drugs have multichannel activity at higher concentrations and therefore lose their proarrhythmic potential, for example quinidine and verapamil (Grace and Camm, 1998; Aiba *et al*., 2005). Points are rounded to the nearest whole number. Data are summed to give a proarrhythmic score (S) at 10-, 30-, 100- and 300-fold the $EFTPC_{max}$ for each drug and these are averaged to give a mean score (mean symbol and standard error: s.e.) for each category at and above the $EFTPC_{max}$ (Table 1). The proarrhythmia score for individual drugs estimates the potential of a drug to induce TdP in humans: the higher the score the greater the likelihood of inducing proarrhythmia. Ketanserin ($n=6$ animals) is used as an example to illustrate how the proarrhythmic score is calculated:

- At the $EFTPC_{max}$ there was one incidence of triangulation (10 points) and one incidence of reverse frequency-dependence (5 points). The score at the $EFTPC_{max}$ is $15/6=2.5$, which is rounded up to 3.
- At 10-fold the $EFTPC_{max}$ there were two incidences of triangulation (20 points), three incidences of reverse frequency-dependence (15 points) and two incidences of instability (40 points). Thus far, the score at 10-fold is $75/6=12.5$. There was also a 10% increase in $MAPD_{90}$ (10 points) giving a score of 22.5. Scores are cumulative so 22.5 plus 2.5 (score at the $EFTPC_{max}$) gives a total score of 25 at 10-fold.
- At 30-fold the $EFTPC_{max}$ there were three incidences of triangulation (30 points), six incidences of reverse frequency-dependence (30 points) and three incidences of instability (60 points). Thus far, the score at 30-fold is $120/6=20$. There were also incidences of EADs and VT (100 points), giving a score of 120. Scores are cumulative so 120 plus 25 (score at 10-fold) gives a total score of 145 at 30-fold.
- At 100-fold the $EFTPC_{max}$ there were three incidences of triangulation (30 points), five incidences of reverse frequency-dependence (25 points) and four incidences of instability (80 points). Thus far, the score at 100-fold is $135/6=22.5$. Scores are cumulative so 22.5 plus 145 (score at 30-fold) gives a total score of 167.5, which is rounded up to 168 at 100-fold.
- At 300-fold the $EFTPC_{max}$, one experiment was automatically terminated because of the high incidence of proarrhythmia, so $n=5$ animals at this concentration. At 300-fold, there were three incidences of triangulation (30 points), five incidences of reverse frequency-dependence (25 points) and two incidences of instability (40 points). Thus far, the score at 300-fold is $95/5=19$. Scores are cumulative so 19 plus 167.5 (score at 100-fold) gives a total score of 186.5, which is rounded up to 187 at 300-fold.

We have estimated that a proarrhythmic score >25 has significant potential to induce TdP in man. This is based on the following: (i) more than one incidence of triangulation, instability, reverse frequency-dependence or a $>10\%$ change in APD_{60} is required to exceed 25 points suggesting that one single event is not sufficient to designate a drug as proarrhythmic; (ii) from the many permutations of TRIad (triangulation, reverse frequency-dependence and instability) elements required to exceed 25 points, greater weight is given to instability, two incidences are >25 points, relative to reverse frequency-dependence or triangulation (two incidences are <25 points); (iii) a 10% increase in APD_{60} alone will not exceed the threshold; (iv) all Category 5 drugs have scores <25 points except where overt proarrhythmias have been observed. In addition, no Category 5 drugs exceed this score at a concentration that was equivalent to 30-fold the $EFTPC_{max}$. This threshold provides an upper limit of safety up to 30-fold the $EFTPC_{max}$ that can be applied to unknown compounds to obtain an estimate of risk and therefore make decisions on the progression of a compound (in combination with other cardiovascular/QT-related assays). A compound incurring a score above this 25-point threshold within a 30-fold margin of the $EFTPC_{max}$ would be unlikely to progress into development. In theory, the threshold could be higher or lower, but this would likely result in a greater number of high-risk compounds going into development or good compounds being discarded. Scores $\geqslant 100$ are clearly torsadogenic. The average proarrhythmic score for each category provides a general proarrhythmic profile for each category (Figure 3). At the EFTCP, Category 1 has the highest score, followed by Categories 2, 3 and close to equal scores for Categories 4 and 5. All but Category 1 drugs are within the 'Safe Zone' (green) at $EFPTC_{max}$. At 10-fold the $EFTPC_{max}$, all scores increase and Category 2 and 3 drugs are no longer considered 'safe', but rather enter the 'Unsafe Zone (red)'. Only Category 4 and 5 drugs remain within the Safe Zone. At 30-fold the $EFTPC_{max}$, scores for all categories increase and only Category 5 drugs are considered safe. At 100-fold the $EFTPC_{max}$ no drugs are considered safe. There is no meaningful score for Category 1 and 2 drugs above 30-fold the $EFTPC_{max}$ because at higher multiples of the $EFTPC_{max}$ most experiments on these drugs were automatically terminated as a result of excessive proarrhythmic activity (see Methods). Figure 3 shows that the results from this study are consistent with the torsadogenic categories outlined by Redfern *et al*. (2003): repolarisation-prolonging drugs and drugs withdrawn or suspended from the market appear far worse than drugs that have no published reports of TdP in humans. The rank order of drugs, as suggested by Redfern *et al*. (2003), is upheld. However, while these analyses show a clear discrimination between Categories 1 and 5, this is not the case between Categories 2, 3 and 4.

Table 1 Summary data from all drugs tested in the rabbit isolated Langendorff heart proarrhythmia model (SCREENIT)

Category/Drug	$EFTPC_{(max)}$ (nm)	$EFTPC_{(max)}$	S	X̄	s.e.	10-fold	S	X̄	s.e.	30-fold	S	X̄	s.e.	100-fold	S	X̄	s.e.	300-fold	S	X̄	s.e.	Overt ProA		1st event
1 Almokalant	150	$MAPD_{60}$	**110**			5T 1R 2I	**146**			— — — —	—			— — — —	—			— — — —	—			**EADs TdP**	**@1**	$MAPD_{60}$
Azimilide	70		0				0			$MAPD_{60}$	10				10			1T 3R	18			EADs	@washout	$MAPD_{60}$
Disopyramide	742		0			$MAPD_{60}$	10				10			1T	15				15					$MAPD_{60}$
DL-sotalol	14733	$MAPD_{60}$	**113**			1T 1R	**115**			2T 1R	**123**			— — — —	—			— — — —	—			**EADs TdP**	**@1**	$MAPD_{60}$ R
Dofetilide	2	1I	7			1T 1R 1I $MAPD_{60}$	**135**			1T	**140**			— — — —	**140**			1T 1R	**155**			**EADs TdP**	**@10**	I
Encainide	61		0			1I	**103**			2R $MAPD_{60}$	**115**			2I	**122**			4T 3I	**138**			**VT**	**@10**	I VT
Ibutilide	140		**103**			2T 1R 1I $MAPD_{60}$	**136**			1T 1I	**166**				**166**			— — — —	—			**EADs TdP**	**@1**	T EADs TdP
Procainamide	54186		**100**	**54**	20	2T 2R 3I $MAPD_{60}$	**125**	**96**	20	2T 1R 2I	**141**	**101**	24	— — — —	—	NA		— — — —	—	NA		**EADs**	**@1**	EADs
2 Astemizole	0.26		0			1T $MAPD_{60}$	**113**			2T	**117**			3T	**127**			— — — —	—			**Proarrhythmia**	**@10**	T $MAPD_{60}$ ProA
Cisapride	4.9		0			1I $MAPD_{60}$	17				**117**			— — — —	—			— — — —	—			**TdP**	**@30**	I
Grepafloxacin	2087		0			1I $MAPD_{60}$	17			2T 1R 1I	**132**			— — — —	—			— — — —	—			**EADs TdP**	**@30**	I $MAPD_{60}$
Terfenadine (1)	9		0				0			1T 1I	**105**			1T	**107**			2T 1R	**111**			**EADs**	**@30**	T I EADs
Terfenadine (2)	9	1I $MAPD_{60}$	13			1T 2R 1I	20			3T 1R 3I	**36**			4T 3R 1I	**48**			4T 3R	**58**					I $MAPD_{60}$
Terodiline	12		**100**	**19**	16		**100**	**45**	20		**100**	**101**	14	$MAPD_{60}$	**110**	NA		1R	**112**	NA		**EADs VF**	**@1**	EADs, VF
3 Aprinidine	239	1T 1R 1I	12			1T 1R 1I	23			1T 1R $MAPD_{60}$	**38**			1T 1R	**53**			1T 2R 1I	**193**			VF	@300	T R I
Azithromycin	421	VF	**100**			1T	**102**			1T 1R 1I	**108**			1R $MAPD_{60}$	**118**			1R	**119**			**VF**	**@1**	VF
Bepridil	330		0				0			$MAPD_{60}$	10			1I	**120**			— — — —	—			TdP	@100	$MAPD_{60}$
Erythromycin	8516	1T 1I	5			1T 1I $MAPD_{60}$	**120**			1T 5R	**126**			4T 5R 2I	**143**			— — — —	—			**EADs**	**@10**	T I
Flecainide	753		0			3T 7R $MAPD_{60}$	**121**			1T 3R 1I $MAPD_{60}$	**128**			3R	**131**			— — — —	—			**EADs**	**@3**	T R EADs
Halofantrine	0.5[a]		0				0			2T $MAPD_{60}$	17			1T 1R	**32**			1T	**37**					$MAPD_{60}$ T
Pimozide	1	1R 1I	4				4			1R $MAPD_{60}$	15			2T 2R 2I	**127**			1T 1R	**129**			EADs TdP VF	@100	R I
Sertindole	1.59		0	**15**	12	2T 1R 2I	22	**49**	19	1T 2R $MAPD_{60}$	**135**	**72**	20	2T 2R 2I	**155**	**110**	15	2T 2R 2I	**165**	**129**	**26**	**EADs TdP**	**@10**	T R I
4 Ciprofloxacin (1)	5281		0				0				0			— — — —	—			— — — —	—					
Ciprofloxacin (2)	5281	1R	1				1				1				1			1I	**4**					R
Clarithromycin (1)	1206		0				0			1R $MAPD_{60}$	11			— — — —	—			— — — —	—					$MAPD_{60}$
Clarithromycin (2)	1206		0				0			1R	1			2T 2I $MAPD_{60}$	21			5T 1I	**133**			EADs	@300	R
Desipramine	108	1T	2			1T	3			1T	**105**			1R	**106**			1T 1R 2I	**133**			**EADs**	**@30**	T
Diphenhydramine	34		0				0			$MAPD_{60}$	10			1R	12			1T 1R	**114**			EADs	@300	$MAPD_{60}$
Domperidone	19	1I	3			1T 1R 1I $MAPD_{60}$	**119**			2T 2R 5I	**141**			3T 2R 2I	**157**			3T 2R 2I	**177**			**EADs TdP**	**@10**	I
Fexafenadine	345		0				0				**100**			1I	**107**			2I $MAPD_{60}$	**123**			**EADs**	**@30**	EADs
Imipramine	106	1I	3			2R	5			2R	**107**			3R $MAPD_{60}$	**119**			1T 3R 1I	**127**			**VF**	**@30**	I
Ketanserin	18	1T 1R	3			2T 3R 2I $MAPD_{60}$	25			3T 6R 3I	**145**			3T 5T 4I	**168**			3T 5R 2I	**187**			**EADs VT**	**@30**	T R
Mibefradil	12		0				0			1R	1			1R	2			1R	**103**			VF	@300	R
Nifedipine	7.7		0				0			2R 1I	10				10				10					R I
Propafenone	241		0			1R $MAPD_{60}$	11			3T 2R	**118**			3T 2I	**129**			1I	**133**			**EADs VT**	**@30**	$MAPD_{60}$ R
Sparfloxacin	1766	1I	3			1T 2I	**112**			1T 1I $MAPD_{60}$	**127**			— — — —	—			— — — —	—			**EADs**	**@10**	I
Ziprasidone	4.1		0	**1**	0.4		0	**18**	10	1I	3	**59**	16		3	**70**	19	2T 2I $MAPD_{60}$	**123**	**114**	16	EADs TdP VF	@300	I
5 Alfuzosin	0.015	1R 1I	4			1R 1I	8			1R 1I	13				**113**			1T 1I $MAPD_{60}$	**128**			EADs	@100	R I
Amlodipine	1.75		0				0				0				0				0					
Amoxicillin	13629		0				0				0				0				0					
Captopril	1726		0				0				0				0				0					
Cetirizine	56		0			1I $MAPD_{60}$	17				17				17			1R	18					$MAPD_{60}$ I
Chlorpheniramine	11	1I	3				3			1I $MAPD_{60}$	17				17			2T 3R 1I	**126**			EADs	@300	I
Cibenzoline	976	1I	3			1R 1I $MAPD_{60}$	18			1T 2R	21			2T 3R 1I	**130**			3R	**133**			VT	@100	I
Diltiazem	122		0				0				0				0				0					
Ebastine	5.1		0				0				0			$MAPD_{60}$	3				3					$MAPD_{60}$
Ketoconazole	177	1I	3			3R 1I $MAPD_{60}$	19			2T 2R	24			3T 2R 1I	**134**			5T 2R 1I	**148**			EADs VT TdP	@100	I
Vardenafil	2159		0			1T 1R	**103**			2T $MAPD_{60}$	**116**			2T	**119**			— — — —	—			**VT**	**@10**	T R VT
Loratidine	0.45	— — — —	—			— — — —	—			— — — —	—				0				0					
Mefloquine	95.2		0			1T 1R	3				3			2T 2R 1I	11			3T 2R 1I -$MAPD_{60}$	21					T R
Moxifloxacin	4.111		0			1I $MAPD_{60}$	**113**			2R 1I	**118**			4T 5R 2I	**136**			5T 5R 3I	**158**			**VT**	**@10**	$MAPD_{60}$ I VT
Nitrendipine	3.02	— — — —	—			— — — —	—				0			$MAPD_{60}$	0			0						
Olanzapine	5.2		0				0				0				0				0					
Paracetamol	13629		0			— — — —	—			— — — —	—			— — — —	—			— — — —	—					
Pentamidine	10		0				0				0				0				0					
Risperidone	1.81	1R 1I	**104**			1T 2R 2I $MAPD_{60}$	**114**			— — — —	**114**			3T 3R 3I	**132**			— — — —	—			**EADs TdP**	**@1**	RI EADs
Tamoxifen	21		0				0			1T 2R	2			$MAPD_{60}$	12			4T 1I	19					T R
Verapamil	81		0			4R	3			1T 5R	9			6R	14			6R -$MAPD_{60}$	19					R
Glucose (1)	4.5[b]		0				0				0				0				0					
Glucose (2)	4.5[b]	1T 1R 1I	6			— — — —	—			— — — —	—			— — — —	—			— — — —	—					T R I
NaCl (1)	0.009[b]		0				0				0				0									
NaCl (2)	4.5[b]	1T 1R 1I	6	**6**	4.5	— — — —	—	**20**	8.8	— — — —	—	**22**	8.8	— — — —	—	**38**	12	— — — —	—	**39**	13			T R I

Proarrhythmia profiles for all drugs are shown. Compounds that are shaded in Category 1 are false negatives, and compounds that are shaded in Category 5 are false positives (see Discussion). The $EFTPC_{max}$ for each drug is shown (Redfern *et al.*, 2003; DrugDex®, 2005). Drug effects are shown relative to the $EFTPC_{max}$ and at 10-, 30- 100- and 300-fold above this value. Proarrhythmia variables are defined as: **T** (triangulation), **R** (reverse frequency-dependence), **I** (instability) and **$MAPD_{60}$** to indicate a 10% increase in **$MAPD_{60}$**. The number of each variable is included. A greater than 10% shortening of $MAPD_{60}$ is indicated as **-$MAPD_{60}$**. The first proarrhythmic variable(s) observed after drug application are listed as the '1st Event'. At each margin a proarrhythmic score (**S**) has been calculated for each drug, and is based on the incidence of proarrhythmia variables and overt proarrhythmia. An average proarrhythmic score (in bold) and standard error of the mean (mean symbol and s.e.) have been calculated for each drug category (these values have been used to construct Figure 3). Scores > 25 are in bold and indicate a torsadogenic potential. Overt proarrhythmia (EAD, VT, TdP and VF) and the margin at which these first occur is also noted; if these occur below 30-fold the $EFTPC_{max}$ they are in bold to highlight their significance. Dashed lines indicate where no data are available. Blank spaces indicate that there was no significant effect. NA = not applicable.

[a]Free plasma concentrations are unknown so the total effective therapeutic plasma concentration has been used.

[b]Concentrations for D-glucose and NaCl were intended to be tested as inert agents, having no pharmacological relevance.

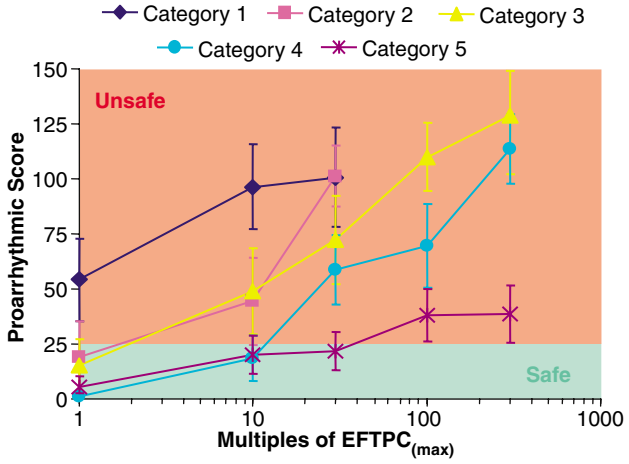

Figure 3 Cumulative average proarrhythmic scores for each drug category at the $EFTPC_{max}$ and above. The cumulative average proarrhythmic score and standard error of the mean (mean symbol and s.e.) for each drug category (values shown in Table 1) at the $EFTPC_{max}$ and above are shown. The data show that scores <25 are safe (Safe Zone, green) and scores >25 are proarrhythmic (Unsafe Zone, red).

Overview of proarrhythmic data

Proarrhythmic profiles for all drugs are shown in Table 1 and a representative example from each category is shown in Figure 4a–f. The series of graphs in Figure 4 include the EFTPC range (vertical dark shading), threshold values for $MAPD_{60}$ and the combined proarrhythmia variables triangulation, reverse frequency-dependence and instability, collectively called 'TRIad elements', per cent change in $MAPD_{60}$, and the per cent TRIad elements against the tested nominal concentration range (see Figure 3 legend for a detailed explanation of % TRIad elements). The results in Table 1 show: (i) Category 1 and 2 drugs have the highest number of proarrhythmia variables and overt proarrhythmia at concentrations of 10-fold or less than the $EFTPC_{max}$; (ii) for Category 3 and 4 drugs, the number of proarrhythmia variables increases with increasing concentration. Drugs from Categories 3 and 4 exhibit a wide range of complex electrophysiological profiles. The highest incidence of proarrhythmia variables tended to be clustered above 30-fold the $EFTPC_{max}$. Results for these intermediate Categories (3 and 4) are positioned between the extremes of Categories 1 and 2, and of Category 5; (iii) drugs in Category 5 show effects, if any, at high multiples of the $EFTPC_{max}$. Category 5 drugs have the lowest incidence of proarrhythmia variables, and when these were noted, they tended to occur as single events at lower $EFTPC_{max}$ multiples, whereas at higher $EFTPC_{max}$ multiples (100- and 300-fold) proarrhythmia variables tended to be clustered. Generally (though see Discussion), overt proarrhythmia occurred infrequently in Category 5 drugs, when they did occur, these were the exceptions and at higher multiples (>100-fold) of the $EFTPC_{max}$; and (iv) D-glucose was used as a negative control substance, there were no significant effects.

In Table 1, drugs are listed in Categories 1–5 according to Redfern *et al*. (2003). Drug effects are shown relative to the maximum $EFTPC_{max}$ and at 10-, 30-, 100- and 300-fold above this value.

Category 1 and 2 drugs

Category 1 drugs include repolarisation prolonging (Class Ia and Class III) antiarrhythmics (which have I_{Kr} block as an integral pharmcacodynamic mechanism and QT prolongation as an intended desirable effect) and Category 2 drugs include those that have been withdrawn or suspended from the market due to an unacceptable risk of TdP. All drugs in Category 1 significantly prolong the $MAPD_{60}$ within 30-fold of the $EFTPC_{max}$. This was not unexpected since Category 1 drugs have APD prolongation as their primary pharmacodynamic mechanism. Figure 4a shows that almokalant causes significant $MAPD_{60}$ prolongation below the therapeutic range, without the appearance of any TRIad elements. Excessive $MAPD_{60}$ prolongation precedes the induction of EADs at 0.045 μM and TdP at 0.45 μM, in all hearts. All three experiments were terminated prematurely due to chaotic activity of TdP at the third concentration, 0.45 μM. Category 2 is represented by grepafloxacin. $MAPD_{60}$ and TRIad elements begin to increase in parallel immediately below the $EFTPC_{max}$ and continue to increase above the $EFTPC_{max}$. Within 30-fold the $EFTPC_{max}$, EADs and TdP were observed. When Categories 1 and 2 are grouped, instability of $MAPD_{60}$ appears to be the most sensitive proarrhythmia variable, often occurring before any other variables. Most drugs in Categories 1 and 2 induce overt proarrhythmia within 30-fold of the $EFTPC_{max}$ (Table 1), exceptions are azimilide, disopyramide and terfenadine (2) – one of the two times terfenadine was tested. Proarrhythmic scores (S) $\geqslant 100$ indicate a high torsadogenic risk (Table 1). High scores are obtained by the occurrence of multiple proarrhythmia variables at a single concentration or by the incidence of overt proarrhythmia. Most drugs in Categories 1 and 2 have scores >100 at or below 30-fold the $EFTPC_{max}$, exceptions are those drugs listed above. Terfenadine (2), with a score >25 at 30-fold the $EFTPC_{max}$, is recognised here as having significant potential to induce proarrhythmia; however, there was no appearance of overt proarrhythmia up to 300-fold the $EFTPC_{max}$. The other drugs, azimilide and disopyramide, may be considered false negatives (see Discussion, False negatives).

Category 3 and 4 drugs

Category 3 drugs are those that have a measurable incidence of TdP in humans or for which numerous case reports exist and Category 4 drugs are those for which there have been isolated reports of TdP in humans. Results from Categories 3 and 4 are the most variable. Drugs in these categories are capable of moving between the categories, particularly as new data become available. Category 3 and 4 drugs are represented by bepridil and clarithromycin (1), respectively (Figure 4c and d). Both drugs show that $MAPD_{60}$ begins to increase before any TRIad elements begin to appear. However, when $MAPD_{60}$ is significantly prolonged there are a significant number of TRIad elements (at about 100-fold the $EFTPC_{max}$) and TdP develops in two of three hearts exposed to bepridil. Clarithromycin (1) causes fewer TRIad elements and no overt proarrhythmia are observed, in spite of $MAPD_{60}$ prolongation; however, it is important to note that clarithromycin (1) was tested at lower concentrations, not

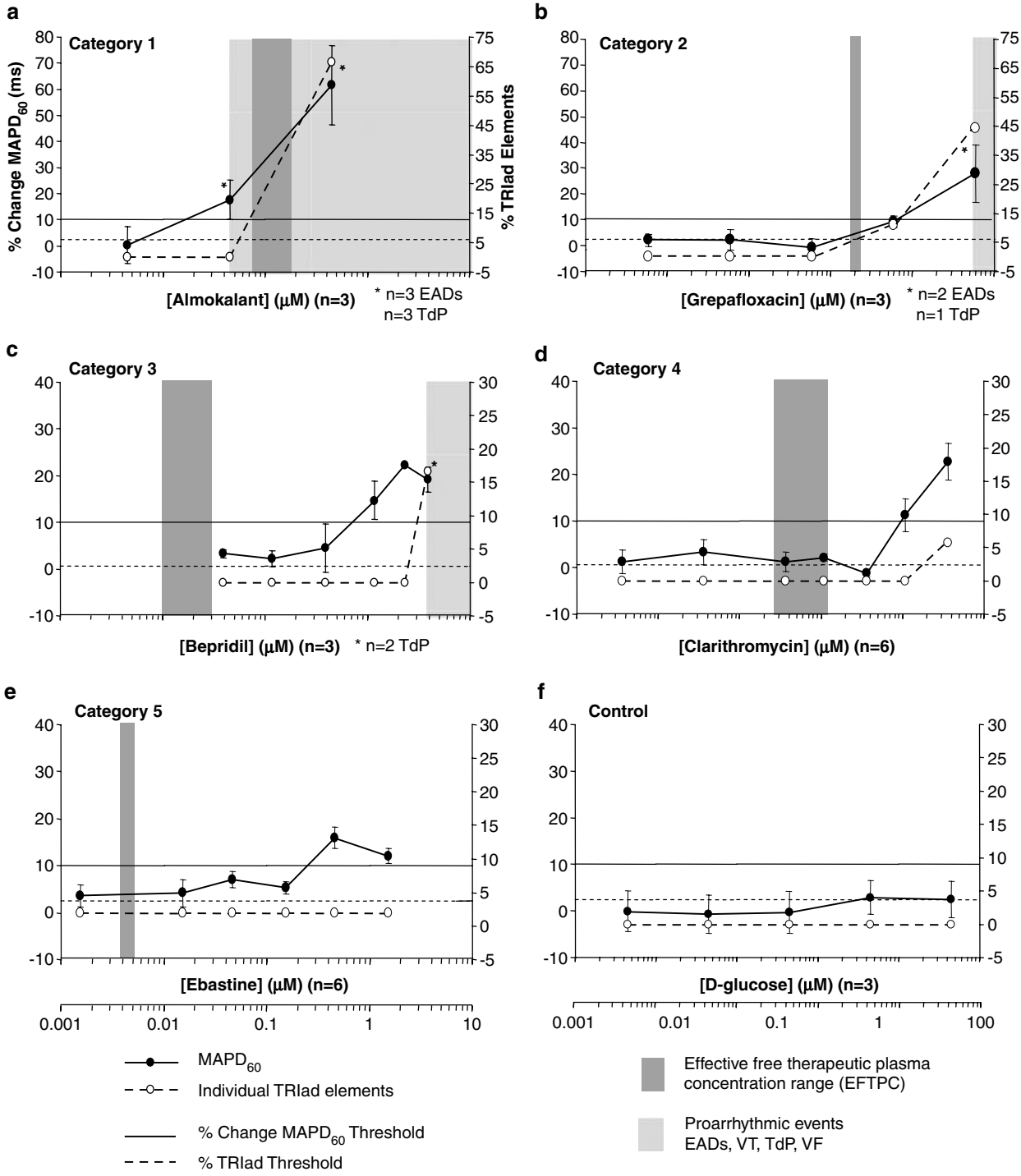

Figure 4 Representative drugs for each drug Category 1–5. The results for a representative drug from each of the five drug categories outlined by Redfern *et al*. (2003) are shown. Each TRIad element, T, R or I, is counted as '1' when a critical level or threshold is attained above control levels. Each TRIad element has its own threshold, previously defined: $T = 29$ ms, $R = 6$ ms, $I = 14$ ms (Hondeghem *et al.*, 2001). The per cent TRIad elements, at a particular concentration, is calculated by summing the number of TRIad elements that occur and dividing this sum by the total number of TRIad elements possible, which is 18 (6 hearts $\times$ 3 (one possibility for each T, R or I) = 18), multiplied by 100. For example, at approximately 10-fold the EFTPC, grepafloxacin induced 1 incidence of instability. The sum of TRIad elements is: 1. Divide this sum by the total number of TRIad elements possible (3 hearts $\times 3 = 9$ and multiply by 100: $(1/9) \times 100 = 11\%$, as shown in (**b**). The threshold for percentage TRIad elements which is greater than the variability for control is 2.5% (Hondeghem *et al.*, 2001). Axes labels in (**a**) apply to all graphs. Almokalant is a representative Category 1 drug. Category 1 drugs include Class Ia and Class III antiarrhythmics. (**b**) Grepafloxacin is a representative Category 2 drug. Category 2 drugs include those drugs that have been withdrawn or suspended from the market due to an unacceptable risk of TdP. (**c**) Bepridil is a representative Category 3 drug. Category 3 drugs are those that have a measurable incidence of TdP in humans or for which numerous case reports exist. (**d**) Clarithromycin is a representative Category 4 drug. Category 4 drugs are those for which there have been isolated reports of TdP in humans. (**e**) Ebastine is a representative Category 5 drug. Category 5 includes drugs for which there have been no published reports of TdP in humans. (**f**) D-glucose has been used as a control substance.

exceeding 30-fold the $EFTPC_{max}$. Clarithromycin was tested a second time but at higher multiples above the $EFTPC_{max}$. Table 1 shows that there is remarkable similarity between the two test datasets for clarithromycin. At 300-fold the $EFTPC_{max}$ clarithromycin (2) shows a marked increase in TRIad elements and EADs. In general, there are few proarrhythmic incidences below 30-fold the $EFTPC_{max}$, whereas the frequency of proarrhythmia variables, and therefore the proarrhythmic score, increases dramatically at 30-fold and above.

Category 5

Category 5 includes drugs for which there have been no published reports of TdP in humans. Category 5 drugs are represented by ebastine. Ebastine causes significant $MAPD_{60}$ prolongation above 100-fold the $EFTPC_{max}$, without any sign of TRIad elements, nor are there any overt proarrhythmia (Figure 4e). These results suggest that $MAPD_{60}$ can be prolonged without inducing proarrhythmia and question the notion of a 'cause and effect' relationship between prolongation and proarrhythmia. The results show that with the exception of three drugs (vardenafil, moxifloxacin and risperidone) the remaining 17 drugs + 2 controls show a very low incidence of proarrhythmia variables and overt proarrhythmia, and therefore have very low proarrhythmic scores at or below 30-fold the $EFTPC_{max}$. Up to 30-fold $EFTPC_{max}$, all scores (with the exceptions noted above) are below 25; the two drugs that achieve scores approaching 25 points induce overt proarrhythmia at the next concentration (100-fold the $EFTPC_{max}$). Many of the proarrhythmic scores remain very low up to and including 300-fold the $EFTPC_{max}$. Vardenafil, moxifloxacin and risperidone show strong proarrhythmic signals at low multiples of $EFTPC_{max}$, these drugs may be considered false positives (see Discussion, False positives).

First proarrhythmic variable

Generally, the proarrhythmia variable that appears most frequently is instability. Proarrhythmia variables seldom occurred in isolation: if one is present, it is highly likely that other proarrhythmia variables will also be observed. It has been suggested that these proarrhythmia variables are not entirely independent and that some kind of synergy exists; however, we have not defined this synergy and therefore have not considered this in our analysis. In general, at least one proarrhythmia variable: an average increase in $MAPD_{60}$ greater than 10%, triangulation, reverse-frequency-dependence or instability preceded overt proarrhythmia. Category 1 and 2 drugs have the highest number of overt proarrhythmia while Category 5 drugs have the least.

hERG (human ether-a-go-go-related gene) margin versus proarrhythmic margin

Figure 5 shows seven drugs from the five torsadogenic categories outlined by Redfern *et al*. (2003). These drugs have (approximately) a 10-fold margin between the hERG IC_{50} and the $EFTPC_{max}$. hERG IC_{50} values were taken from the literature (Redfern *et al*., 2003). The graph shows that at the therapeutic dose the proarrhythmic scores for all drugs, except procainimide (Category 1), is very low. Procainimide has a score of 100 at the $EFTPC_{max}$ due to the incidence of EADs. Proarrhythmic scores begin to increase for all drugs at higher concentrations/greater margins above the $EFTPC_{max}$. At 30-fold $EFTPC_{max}$, six of the seven drugs attain very high proarrhythmic scores. Ketoconazole, a Category 5 drug, has a relatively low proarrhythmic score suggesting that it is associated with less proarrhythmic risk than the drugs from Categories 1–4. While there appears to be a clear separation between Category 1 and 5 drugs at 30-fold the $EFTPC_{max}$, there is no clear separation between drugs in Categories 2, 3 or 4. Above 100-fold $EFTPC_{max}$, all drugs are shown to have high proarrhythmic scores. It is not surprising that for many drugs the proarrhythmic score is high at 300-fold the $EFTPC_{max}$ since the scores are cumulative. This may be misleading because at higher concentrations drugs tend to have activity at multiple ion channels, which may afford some protection against the prolonging effect of hERG channel block, as is the case for verapamil (Aiba *et al*., 2005). These data show that this proarrhythmia model is capable of separating proarrhythmic from non-arrhythmic drugs when discrimination between drugs on the basis of the hERG $IC_{50}/EFTPC_{max}$ margin is not possible.

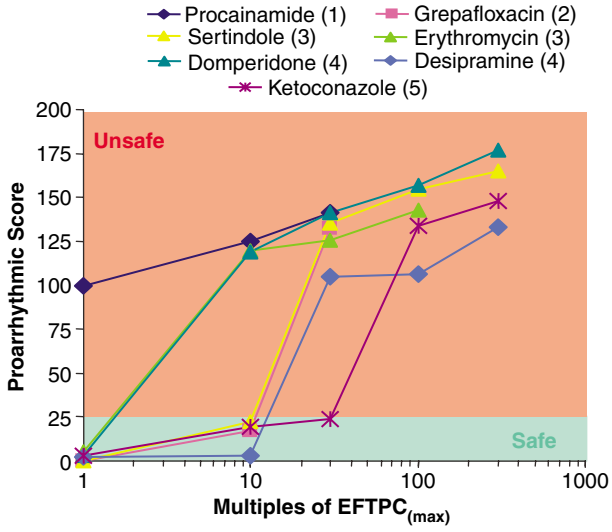

Figure 5 Proarrhythmic scores for drugs with a 10-fold hERG IC_{50}/ $EFTPC_{max}$ ratio. Seven drugs that span the five torsadogenic categories outlined by Redfern *et al*. (2003) are shown. These drugs have an approximate 10-fold margin between the hERG IC_{50} and the $EFTPC_{max}$ (Redfern *et al*., 2003; DrugDex®, 2005). Bracketed numbers following the compound name refer to the category number as defined by Redfern *et al*. (2003).

Discussion and conclusions

The female rabbit isolated Langendorff heart proarrhythmia model (SCREENIT) was used to determine the proarrhythmic potential of a range of marketed drugs or drugs intended for market with varying torsadogenic risk. Drugs were

categorised according to their torsadogenic profile as suggested by Redfern *et al*. (2003). Category 1 and 2 drugs are highly associated with clinical TdP, and were found to have a small margin (<30) between the hERG/I_{Kr} IC_{50} and the $EFTPC_{max}$ (Redfern *et al*., 2003). We found that many drugs from Categories 1 and 2 had high proarrhythmic scores within low margins (<30-fold) of the $EFTPC_{max}$. From our data it could be predicted that these drugs would have a high propensity to cause TdP (Figure 3). Conversely, for Category 5 drugs, which have no published reports of TdP in humans, we found that most of these drugs were free of proarrhythmic risk non-clinically, particularly below and including 30-fold $EFTPC_{max}$ (Table 1 and Figure 3), but in many cases up to 300-fold $EFTPC_{max}$. The proarrhythmic profile associated with each drug category may help to determine the proarrhythmic potential of new chemical entities, for example, there will be different development outcomes for a compound depending on whether it displays a Category 5 or Category 1-like profile (Figure 3). These data and the proarrhythmic scores calculated for each drug category at the $EFTPC_{max}$ and above provide a framework to aid decision-making on the proarrhythmic liability associated with new chemical entities. Furthermore, these data suggest that this *in vitro* non-clinical proarrhythmia model can predict clinical outcome, with few exceptions.

Prolongation: proarrhythmic or protective?

A greater than 10% prolongation of $MAPD_{60}$ has been used as a proarrhythmic variable in this study. Our data, for example see ebastine (Figure 4e), show that prolongation *per se* is not necessarily proarrhythmic. Our data support the view that other factors, besides prolongation, contribute to a proarrhythmic substrate, and that these other factors (triangulation of the MAP, reverse frequency-dependence, beat-to-beat variability of MAPD, quantified as instability, and probably other variables not investigated in this study such as spatial dispersion of repolarisation) are likely to play a larger role in determining the potential for proarrhythmia in the clinic (Hondeghem *et al*., 2001; Belardinelli *et al*., 2003). In combination, these factors more accurately predict clinical outcome than any single variable.

In our hands, prolonged repolarisation can, but does not always, give rise to the development of EADs (similar to clinical findings) particularly in Purkinje fibres, but more so when prolongation is exaggerated by reverse frequency-dependence (Abi-Gerges *et al*., 2004). It has been suggested, however, that prolongation is only deleterious when it is complicated by triangulation, reverse frequency-dependence or instability otherwise prolongation is protective (Shah and Hondeghem, 2005). Our experience dictates that we need to consider excessive prolongation as associated with proarrhythmia, as did Champeroux *et al*. (2005). Our data show that MAPD prolongation is associated with the incidence of proarrhythmia, but it is a rather imprecise marker of proarrhythmia, particularly if it is used as the sole predictor of impending proarrhythmia. This suggests that measurement of the QT interval in the clinic, as the sole predictor of TdP, is a simple and, at best, gross indicator of TdP liability.

False negatives and false positives

To determine the percentage of false negatives and false positives we considered that drugs in Categories 3 and 4 (numerous or isolated reports of TdP) were unlikely to cause consistent proarrhythmia, and results from these categories may be quite variable. We therefore concluded that 'true' false negatives would be drugs from either Categories 1 or 2 that showed no torsadogenic propensity (proarrhythmic score <25) below 30-fold the $EFTPC_{max}$. Similarly, 'true' false positives would be those drugs from Category 5 (no published reports of TdP in humans) that showed a clear torsadogenic liability (proarrhythmic score >25) at 30-fold or below the $EFTPC_{max}$.

False negatives

Two drugs from Categories 1 and 2 were not identified by SCREENIT as torsadogenic or having the potential to pose a proarrhythmic risk to humans (Table 1). These were azimilide and disopyramide. Azimilide is a class III anti-arrhythmic that blocks both slow and fast repolarizing K^+ currents (Chen *et al*., 2002) and was identified in the ALIVE trial (2001) (Camm *et al*., 2004) as having a small torsadogenic risk at a daily dose of 100 mg. Interestingly, the torsadogenic risk for azimilide is significantly lower than that for other class III anti-arrhythmics (e.g. sotalol, DIAMOND-MI trial; Lehmann *et al*., 1996). We found that while azimilide did not cause any untoward effects during perfusion at any concentration, it was associated with EADs on washout. This suggests that azimilide may pose a risk between doses/treatments and on drug withdrawal rather than when the drug has reached a steady-state. This is not uncommon and has been well documented for other drugs such as quinidine (Kim and Benowitz 1990; Grace and Camm, 1998). Nevertheless, it was expected that at 300-fold $EFTPC_{max}$ there would have been signs of proarrhythmia. Our finding that there were no significant proarrhythmic indications suggests that azimilide's proarrhythmic potential was not detected using the SCREENIT protocols described here. Disopyramide is a class Ia antiarrhythmia agent that blocks I_{Na} as well as I_{Kr} (Carmeliet, 1993; Virag *et al*., 1998), I_{K1}, I_{to} (Coraboeuf *et al*., 1988; Martin *et al*., 1994), I_{KATP} (De Lorenzi *et al*., 1995) and I_{KACh} (Watanabe *et al*., 1997). Disopyramide has been reported to cause TdP but only in combination with at least one other risk factor, for example, concomitant therapy with CYP3A4 inhibitors (clarithromycin) (Choudhury *et al*., 1999) or quinidine (Kim and Benowitz, 1990), acute hepatocellular dysfunction (Schattner *et al*., 1989), an underlying genetic mutation (abnormal K^+ channel resulting in congenital bradycardia) (Ohe *et al*., 1992), age or female gender (De Bruin *et al*., 2005). Our results show that disopyramide has no proarrhythmic liability and in contrast, caused MAP squaring (data not shown), an additional putative anti-arrhythmic effect (Hondeghem *et al*., 2001). This discrepancy between clinical and non-clinical data may be explained by the presence of pre-existing conditions or concomitant therapies received by patients prior to administration of disopyramide. However, with the incidence of case reports using the reporting odds ratio (De Bruin *et al*., 2005), it is still surprising that

disopyramide has surfaced as a safe anti-arrhythmic in the SCREENIT assay. It is unclear, whether this is a true *false negative* or a result of a lack of other confounding factors that are not present in this nonclinical model using a 'healthy heart'. The use of pathological proarrhythmia models (Thomsen *et al*., 2003) that incorporate specific risk factors may help to address this additional layer of complexity that arises in the clinic.

While it may be worrying that this study shows two examples of false negatives, it is worth considering that results from this assay would not stand in isolation but would form part of an integrated cardiovascular risk assessment. These results would be considered in conjunction with results from the core battery of cardiovascular safety assays and possibly those from follow-up and/or supplemental studies.

False positives

There were three drugs in Category 5 that SCREENIT identified as having a significant proarrhythmic risk (Table 1), in contrast to clinical findings. These were vardenafil, moxifloxacin and risperidone. Vardenafil, a PDE-5 inhibitor, has been shown to cause a small increase (5–10 ms) in corrected QT interval (QTc) (Morganroth *et al*., 2004) but to date there have been no published cases of TdP with this drug. Moxifloxacin is a fluoroquinolone antibiotic associated with QT prolongation (Oliphant and Green, 2002). It has been recommended as a positive control by regulatory authorities to evaluate the sensitivity of clinical studies to detect small but significant increases in the QT interval (Anon, 2005b). Clinical studies show a clear QTc prolongation with moxifloxacin, yet there are no published reports of TdP (Andriole *et al*., 2005). However, there have been a number of unpublished reports (approximately 1 report per million subscriptions) to the Federal Drug Administration in the USA of torsadogenic activity induced by moxifloxacin. In a non-clinical assessment (Chen *et al*., 2005), an arrhythmogenic potential of moxifloxacin was observed at approximately 18-fold $EFTPC_{max}$, and they concluded that moxifloxacin appeared to have a high risk of inducing TdP. These results are not dissimilar from our findings in which moxifloxacin was found to pose a proarrhythmic risk at 10-fold $EFTPC_{max}$. In a retrospective analysis of *in vitro*, *in vivo* and clinical findings, moxifloxacin was found to have the potential to prolong QT and cause non-clinical TdP, however, by 2002 there were still no 'published' reports showing that moxifloxacin caused TdP in the clinic (Webster *et al*., 2002). Clearly non-clinical assays predicted a proarrhythmic risk associated with moxifloxacin, including the assay used here: the rabbit Langendorff heart proarrhythmia assay. In the fullness of time, that is time in the marketplace, one may speculate that moxifloxacin would have eventually been withdrawn or suspended from the market due to an unacceptable risk of TdP. In this case, moxifloxacin would have to be re-categorised as Category 2, rather than Category 5, thus providing an explanation for the current apparent disparity between clinical and non-clinical findings.

Risperidone is an antipsychotic drug and a potent I_{Kr} blocker (Drolet *et al*., 2003). Prolongation of the QRS and QTc intervals have been reported to occur following two cases of presumed risperidone overdose (Vieweg, 2003). In addition, one case of sudden cardiac death has been reported in a 34-year-old white woman with no history of cardiac disease. Although other possibilities exist, risperidone cannot be ruled out as the cause of this fatal episode. One hundred and forty three case reports of suspected cardiovascular (cardiac arrest, sudden death, TdP, VF or VT) adverse drug reactions were received by the WHO for risperidone (De Bruin *et al*., 2005), indicating that this drug does not belong in Category 5 of the Redfern categorisation. That a 're-categorisation' is required for some drugs in the Redfern categories is not surprising as Redfern *et al*. (2003) did not have access to the WHO database, but rather compiled and categorised the list of drugs according to publications (i.e. published articles via Medline). The results presented here suggest that the *in vitro* proarrhythmia assay we have used would have predicted the torsadogenic risk posed by clinical use of risperidone. Based on these clinical and non-clinical data, we suggest that risperidone is more accurately described as a Category 4 drug of the Redfern categories (Redfern *et al*., 2003) and has been accurately assessed by SCREENIT.

The Redfern categorisation of drugs (Redfern *et al*., 2003) has been developed relative to current clinical experience and given the rarity of TdP, it is clear that drugs will shift between categories and some overlap is likely. This fluidity between categories (Redfern *et al*., 2003) depends on additional data that comes to light with increasing time in the marketplace and the increasing number of prescriptions, for example, sertindole which was voluntarily withdrawn from the marketplace in 1998 (Category 2) but was reintroduced in 2002 on the basis on new safety data (Category 3) (Matz, 2005).

The low percentage of false negatives (3%: azimilide and disopyramide) and false positives (2%: vardenafil, excluding risperidone and moxifloxacin), albeit to the exclusion of Category 3 and 4 drugs, suggests that this assay adds value to the non-clinical QT/TdP assessment of compounds in early discovery. Using this assay as part of an integrated cardiovascular risk assessment, very few compounds would enter a drug development programme without the proarrhythmic risk being identified and conversely, very few truly safe compounds would be discarded.

Reproducibility

Reproducibility within this validation study. Drugs or control substances that were tested in this study in duplicate include terfenadine, ciprofloxacin, clarithromycin, glucose and NaCl. The first set of results for terfenadine show EADs at 30-fold $EFTPC_{max}$, and therefore a high proarrhythmic score is calculated at this margin and higher. Entirely consistent with the first set of results, the second set of results revealed a significant number of proarrhythmia variables again at 30-fold and higher, producing a significantly high proarrhythmic score at 30-fold $EFTPC_{max}$. These results signal a significant likelihood of proarrhythmia for terfenadine at 30-fold $EFTPC_{max}$ and higher, consistent with clinical experience where the cardiovascular risk increases in the

presence of metabolic inhibitors. Ciprofloxacin and clarithromycin are Category 4 drugs. For both drugs, the first and second sets of results are very similar. Although different proarrhythmia variables may have been observed these were not frequent enough to significantly elevate the proarrhythmic score for either drug, up to and including 100-fold $EFTPC_{max}$. Proarrhythmic effects were observed in the presence of clarithromycin (2) at 100-fold $EFTPC_{max}$ resulting in a proarrhythmic score close to 25 points, indicating the potential for significant proarrhythmic activity. This proarrhythmic potential was revealed at 300-fold $EFTPC_{max}$ where clarithromycin produced two runs of EADs. These data suggest that the presence of a significant number of proarrhythmia variables (proarrhythmic score >25 points) heralds the onset of significant proarrhythmic potential. Control substances, glucose and NaCl, also showed remarkable similarity between the two sets of data. The second series of experiments revealed a few proarrhythmia variables for both glucose and NaCl, but these did not amount to a significant proarrhythmic score (Table 1), nor were these effects considered to be drug related. These results reveal that this *in vitro* proarrhythmia assay produces results that are clinically accurate and indeed reproducible.

Reproducibility between validation studies. Some of the drugs tested in this study were also tested in independent validation studies commissioned by two pharmaceutical companies (Hondeghem and Hoffman, 2003; Hondeghem *et al*., 2003). Table 2 includes drugs that were tested in at least one of these two other studies. Comparisons have been made at single concentrations where these data are available (Hondeghem and Hoffman, 2003; Hondeghem *et al*., 2003). Proarrhythmic scores have been calculated at the single concentrations (only data available) presented in Table 2, thus these values may differ from those in Table 1 where the scores are cumulative across the concentration range tested. Dl-Sotalol, ibutilide, bepridil, sertindole and ziprasidone showed very similar results between our study and Study 1. Terfenadine (1) showed an excellent concordance with all three studies. However, terfenadine (2) did not emerge as particularly proarrhythmic in Study 2 nor in our study; however, in our study the cumulative rather than single proarrhythmic score at 30-fold $EFTPC_{max}$ was 36, indicating significant proarrhythmic potential at this concentration. Terfenadine (2) in Study 2 was tested at five concentrations yet it was impossible to determine a proarrhythmic score except at the maximum test-concentration (information not present in publication), which may underestimate its proarrhythmic potential. There may also be slight differences in experimental methodology between the studies. For instance, our study introduced an irregular stimulation protocol (see Methods, Experimental protocol), which places an additional torsadogenic stress on the heart due to the short-long-short intervals. This analysis suggests that there may be value in considering effects at previous concentrations and that taken together these effects may be additive and more predictive of clinical outcome. Results for cisapride were consistent across studies; cisapride was clearly proarrhythmic at 30-fold the $EFTPC_{max}$ in our study and in Study 1. In our study, erythromycin and flecainide were identified as being proarrhythmic; however, these drugs appeared to be without significant proarrhythmic liability in Studies 2 and 1, respectively. Neither Study 1 nor 2 included an irregular stimulation protocol, suggesting that this may be an important additional stress that can help to determine proarrhythmic potential. It is noteworthy that in Study 2, only three hearts were used to test erythromycin, suggesting that more replicates may be required to detect a 'real' change in the proarrhythmic parameters measured. However, only three hearts were required to show the torsadogenic liability of flecainide in our study, whereas six hearts used in Study 1 failed to show any proarrhythmic potential, again suggesting the value of long–short–long stimulation sequences. Nevertheless, it is remarkable that while the identity of flecainide remained unknown to the investigator, it was correctly identified as a 'very potent class I [antiarrhythmic] with slow kinetics = Ic, later also an increase in APD = Ia' (Hondeghem *et al*., 2003). Diltiazem and verapamil were without proarrhythmic potential in all studies.

This cross-study analysis shows a good degree of similarity among all three studies. A number of reasons have been proposed to explain some of the discrepancies among these studies; these include the effect of serial increases in concentration versus effects at a single test-concentration (terfenadine 2), group size (erythromycin) and assumptions regarding data from Studies 1 and 2, for example, the assumption that TRIad effects occurred at the highest test concentration since it was impossible to define TRIad effects at previous concentrations. Other discrepancies may be a result of changes in the SCREENIT model/methodology. The previous validation studies (Studies 1 and 2) were published in 2003 and employed SCREENIT version 6 (SCREENIT.6) (Hondeghem and Hoffman, 2003; Hondeghem *et al*., 2003). Our study, conducted between 2002 and 2005 used SCREENIT.6 Class III, which was an enhanced version of SCREENIT.6 specifically designed to investigate drug-induced Class III antiarrhythmic effects. The Class III version utilises irregular, random frequency stimulation at each concentration as a further intensive test of a drug's safety. This regime may detect a drug's ill effects at lower concentrations than what otherwise might be detected with regular stimulation protocols and additionally mimics the short–long–short sequence that so often precedes TdP in the clinic (Yap and Camm, 2003).

Other models or methods of determining proarrhythmic risk. In the past 5 years, a rapidly increasing number of models or methods for determining a compound's potential to induce proarrhythmia, in particular drug-induced TdP, have been developed, published and reviewed (for reviews see Lawrence *et al*., 2005; Thomsen *et al*., 2006). While these reviews detail the individual strengths and weaknesses associated with each approach, it is worth noting that MAP recordings from the isolated heart provide information on global repolarisation disturbances, not only MAP duration. This is in contrast to the Purkinje fibre assay, which provides information on APD prolongation, triangulation and reverse frequency-dependence, but cannot provide data on instability or more importantly global repolarisation. In the Purkinje fibre assay, the protective effects conferred by block of an inward current, in addition to hERG channel block, may mask large

Table 2 Reproducibility between validation studies

Category/Drug	*Margin to EFTPC*	*AstraZeneca*							*Study 1*							*Study 2*						
		T	*R*	*I*	*MAPD$_{60}$*	*ProA*	n	*Score*	*T*	*R*	*I*[a]	*MAPD60*	*ProA*	n	*Score*	*T*	*R*	*I*	*MAPD$_{60}$*	*ProA*	n	*Score*
1 dl sotalol	0.30		1R		MAPD$_{60}$	**EADs, TdP**	3	**111**		R			**EADs**	6	**101**							
1 Ibutilide	0.30	1T				**EADs, TdP, VF**	3	**103**	T	R	I	MAPD$_{60}$	**EADs**	6	**116**							
2 Cisapride	30			11	MAPD$_{60}$	**TdP**	3	**117**			I	MAPD$_{60}$	**EADs VT**	6	**113**							
2 Terfenadine (1)	30	1T		11	MAPD$_{60}$	**EADs**	6	**115**	I				**EADs, VT**	8	**103**	2T				VT	3	**107**
2 Terfenadine (2)	30	3T	1R	31			6	16										11			3	*7*
3 Flecainide	3	3T	7R		MAPD$_{60}$	**EADs**	3	**132**				MAPD$_{60}$		6	10							
3 Erythromycin	30	1T	5R		MAPD$_{60}$	**EADs**	6	**116**										11			3	*6*
3 Bepridil	300			11	MAPD$_{60}$	**TdP**	3	**117**				MAPD$_{60}$	**VT, VF**	2	**110**							
3 Sertindole	300	2T	2R	21	MAPD$_{60}$	**EADs, TdP**	6	**121**	T		I	MAPD$_{60}$	**EADs**	2	**125**							
4 Ziprasidone	300	2T		21	MAPD$_{60}$	**EADs, TdP, VF**	6	**120**		R	I	MAPD$_{60}$	**EADs, VT**	2	**123**							
5 Diltiazem	30						3	0						6	0							
5 Verapamil	30	1T	5R				6	6		R				6	5				-MAPD$_{60}$		3	0

The table compares results from our study with independent validation studies commissioned by two pharmaceutical companies (Hondeghem and Hoffman, 2003; Hondeghem *et al*., 2003). Proarrhythmic scores have been calculated at single concentrations and thus may differ from those in Table 1. Nine drugs: dl-sotalol, ibutilide, terfenadine (1), cisapride, bepridil, sertindole, ziprasidone, diltiazem and verapamil, show very good reproducibility between studies. Three drugs, terfenadine (2), erythromycin and flecainide show discrepancies between the studies. Erythromycin and flecainide were shown to be proarrhythmic in our study and relatively benign in Study 2 and 1, respectively. See Table 1 legend for details of table symbols. Blank spaces indicate where no data are available.
[a]The number of TRIad elements at each concentration is unknown.

repolarisation differences in different regions of the heart and declare a potentially torsadogenic compound to be 'safe' (Gintant *et al*., 2001). Interestingly, Champeroux *et al*. (2005) have developed a complex and elegant algorithm from Purkinje fibre data, based on an increase in APD_{90}, reverse frequency-dependence, triangulation or effects on action potential upstroke conduction velocity, to predict proarrhythmic risk. The Purkinje fibre assay undoubtedly holds merit for determining the impact of multichannel block on action potential repolarisation; however, the isolated heart assay may provide additional information on global repolarisation disturbances.

Can a proarrhythmia assay distinguish between drugs with the same hERG IC_{50}/EFTPC$_{max}$ ratio? Can a proarrhythmic safety margin be identified?. Our data show that at 30-fold $EFTPC_{max}$, there is a clear separation of effects between the Category 5 drug, ketoconazole, and drugs from Categories 1–4 (Figure 5), even though these drugs all have a 10-fold margin between the hERG IC_{50} and $EFTPC_{max}$. The variable proarrhythmic scores among drugs may reflect differences in their ion channel selectivity. A selective hERG channel blocker is likely to have a higher proarrhythmic score than a mixed channel blocker since the prolongation induced by block of outward (hERG) current may be counterbalanced by block of inward (e.g. Ca^{2+} and/or Na^{+}) current; therefore, providing some protection against excessive $MAPD_{60}$ prolongation, driving forces for EAD development and also against disperison of repolarisation. These data suggest that: (i) this rabbit Langendorff heart proarrhythmia assay can distinguish proarrhythmic from non-proarrhythmic drugs that have the same hERG IC_{50}/$EFTPC_{max}$ margin and (ii) a margin of 30-fold between significant proarrhythmic events (i.e. a high proarrhythmic score) and the $EFTPC_{max}$ is critical to ensure human safety. This proarrhythmia assay proves to be particularly valuable in discriminating between drugs with similar *in vitro* electrophysiological profiles (same hERG IC_{50}/$EFTPC_{max}$ ratio) where other assays fail.

The delineation of a 30-fold margin is not unique to this data set. A 30-fold safety margin or therapeutic window between the hERG IC_{50} and $EFTPC_{max}$ was suggested from two independent retrospective studies looking at the relationship between non-clinical cardiovascular assessment and clinical outcome for large sets of marketed drugs; 28 (Webster *et al*., 2002) and 53 (Redfern *et al*., 2003). A third study also noted that drugs with $\leqslant$30-fold hERG IC_{50} to $EFTPC_{max}$ had a three to four times stronger association with serious ventricular arrhythmias and sudden death compared with drugs which had a $>$30-fold margin above the therapeutic level (De Bruin *et al*., 2005). Clearly, according to these studies, it is not coincidental that many Category 5 drugs are not proarrhythmic since most have a $>$30-fold margin between the hERG IC_{50} and $EFTPC_{max}$ and therefore are not strongly associated with serious proarrhythmia.

Non-clinical value and clinical concordance. Most drugs with very high proarrhythmic scores are those which showed at least one incidence of overt proarrhythmia (EADs, VT, VF or TdP); however, a high proarrhythmic score can also be achieved by the occurrence of multiple proarrhythmia variables (10% increase in the average $MAPD_{60}$, triangulation, reverse frequency-dependence and instability) at a single concentration. These proarrhythmia variables tend to occur before overt proarrhythmia, suggesting that they can be used as markers signalling the potential onset of a proarrhythmic event. Non-clinical proarrhythmia variables/markers do not have an easily identifiable clinical correlate. While QTc is a poor surrogate for TdP (Thomsen *et al*., 2005), nonetheless it is significantly associated with serious cardiovascular adverse events, including TdP (De Bruin *et al*., 2005). It is interesting to note, however, that in 1993, Schwartz *et al*. suggested that clinicians would be well advised to consider not only QT/QTc prolongation but also T-wave morphology changes, including changes in the peak and width of the T-wave. Indeed, clinical correlates of non-clinical observations of APD triangulation and instability

would be seen as changes in T-wave morphology, such as widening and flattening of the T-wave (Shah and Hondeghem, 2005). Instability of APD, or rather beat-to-beat changes in APD, may be correlated with beat-to-beat changes in the T-wave, such as T-wave alternans (Fossa *et al*., 2004; Thomsen *et al*., 2005) or aperiodic T-wave changes (Malik, 2005) and reverse frequency-dependence can be evaluated at fast heart rates (during exercise) and slow heart rates (during sleep). Future clinical studies may reveal if such ECG-related markers as shown in non-clinical models are also of clinical significance and thus can add to a better safety evaluation in early developmental studies. In fact, a small clinical study on the selective I_{Kr} blocker almokalant, published almost 10 years ago, shows that this may be the case (Houltz *et al*., 1998).

Limitations

There are several weaknesses inherent in an *in vitro* isolated heart model. These weaknesses relate to the choice of sex and species used and their relevance to the general human population (too sensitive or not sensitive enough). An isolated heart model, in contrast to an intact heart model, excludes effects due to plasma protein binding, the effects of metabolites, and neuronal and hormonal influences. It is important to note that calculations of safety margins, as used here, can be greatly influenced by the technical aspects of estimations of plasma protein binding and the presence of possible concentration-dependent protein binding, especially if the degree of protein binding is high. Also, it is often noted that TdP most often occurs in individuals with other complicating factors (e.g. liver dysfunction). Thus, a pathological model rather than a healthy heart model may be a more appropriate model from which to investigate the development of TdP. For these reasons, additional tests in other animal models are warranted, thus underscoring the importance of an integrated QT/TdP risk assessment. Experiment-specific weaknesses include the use of only two MAP electrodes, one epicardial and one subendocardial. This means that proarrhythmic activity remote from the recording electrodes may propagate around the heart before being detected locally, and therefore may lead to an underestimation of proarrhythmic activity. The 10–15 min exposure time may be insufficient for some drugs to equilibrate and therefore effects may be underestimated. The concentration of the drug in the perfusates was not analysed so drug concentrations may be less than intended, a consequence of which would be an underestimation of effects at a given margin above the $EFTPC_{max}$. In these experiments, the extent of cardiac accumulation was not determined. The parameters used to define proarrhythmic risk (triangulation, reverse frequency-dependence, instability and 10% $MAPD_{60}$ prolongation) have been treated as discontinuous variables, whereas in fact, each is continuous. The various degrees of severity for each variable have not been determined, nor has this been considered in calculations of the proarrhythmic score. Finally, the SCREENIT model is not universally available and until the results from this study and other studies can be reproduced and interpreted in a variety of laboratories, in academia and industry, there will inevitably be some (regulatory) reluctance to accept the predictive value of this kind of model.

Conclusion

Fifty-five marketed drugs or drugs intended for market, three replicates and two controls were tested in a blinded manner in the rabbit isolated Langendorff heart assay (SCREENIT). Results have been analysed independently, without benefit from experts on this model. When the results are taken together, in the form of a proarrhythmic score, these can predict clinical outcome, particularly for drugs associated with either very high or very low torsadogenic risk. Between these extremes the model is less precise. Nonetheless, the model is particularly useful where compounds have small margins between the hERG IC_{50} and the predicted $EFTPC_{max}$, or have effects on QT prolongation yet may not be torsadogenic. In particular, the model could be used for compounds targeting life-threatening diseases where on balance a cardiovascular risk may pose less of a concern: that is to say, it is possible to accept a certain amount of risk if the benefit is high relative to the impact of disease progression. The model is also able to help define a safety margin relative to the $EFTPC_{max}$ above which adverse effects may first appear in humans. This study confirms that a 30-fold margin between effects in this assay (incidence of proarrhythmia variables) and the $EFTPC_{max}$ is sufficiently stringent to provide confidence to progress a new chemical entity, but without incurring the risk of eliminating potentially beneficial drugs. A 30-fold margin (between the hERG IC_{50} and the $EFTPC_{max}$) has been shown previously to provide an acceptable degree of safety (Webster *et al*., 2002; Redfern *et al*., 2003; De Bruin *et al*., 2005). The clinical relevance coupled to the good degree of reproducibility suggests that the rabbit Langendorff heart proarrhythmia model is a robust and reliable assay that can add value to an integrated QT risk assessment.

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Conflict of interest

The authors state no conflict of interest.

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