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Blinded validation of the isolated arterially perfused rabbit ventricular wedge in preclinical assessment of drug-induced proarrhythmias

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Abstract

BACKGROUND—The development of preclinical models with high predictive value for the identification of drugs with a proclivity to induce Torsade de Pointes (TdP) in the clinic has long been a pressing goal of academia, industry and regulatory agencies alike. The present study provides a blinded appraisal of drugs, in an isolated arterially-perfused rabbit ventricular wedge preparation, with and without the potential to produce TdP.

METHODS AND RESULTS—Thirteen compounds were tested for their potential for TdP using the rabbit left ventricular wedges. All investigators were blinded to the names, concentrations and molecular weights of the drugs. The compounds were prepared by the study sponsor and sent to the investigator as 4 sets of 13 stock solutions with the order within each set being assigned by a random number generator. Each compound was scored semi-quantitatively for its relative potential for TdP based on its effect on ventricular repolarization measured as QT interval, dispersion of repolarization measured as T_{p-e}/QT ratio and early afterdepolarizations. Disclosure of the names and concentrations after completion of the study revealed that all compounds known to be free of TdP risk received a score of less or equal to 0.25, whereas those with known TdP risk received a score ranging from 1.00 to 7.25 at concentrations less than 100X their free therapeutic plasma C_{max} .

CONCLUSIONS—Our study provides a blinded evaluation of the isolated arterially-perfused rabbit wedge preparation demonstrating both a high sensitivity and specificity in the assessment of 13 agents with varying propensity for causing TdP.

Keywords

Long QT Syndrome; Arrhythmia; Ion Channels; Myocytes; Sudden Cardiac Death

Introduction

It has been recognized for more than a half century that use of certain drugs is associated with the development of life-threatening ventricular arrhythmias. The classic example is “quinidine syncope”. Patients taking quinidine for treatment of atrial fibrillation may have recurrent syncope or sudden cardiac death resulting from a particular form of polymorphic ventricular

tachycardia known as torsade de pointes (TdP).¹ Drug-induced TdP in the clinic, particularly by non-cardiac agents, has resulted in relabelling of a number of drugs for restricted use and the removal of others from the market in recent years. It has also led to a more rigorous review of new drugs by regulatory agencies to determine the potential of such drugs to induce TdP in humans.² The development of a preclinical model with high predictive value for the identification of drugs with a TdP liability in the clinic has long been a pressing goal of academia, industry and regulatory agencies alike. The problem has been validation, that is, providing the predictive value of any preclinical model has been difficult.

TdP generally occurs under conditions of delayed ventricular repolarization or QT prolongation, almost always associated with inhibition of the rapidly activating delayed rectifier potassium current (I_{Kr} , or *hERG* current). As a consequence, regulatory guidelines call for new drug candidates to be tested for I_{Kr} or *hERG* current inhibition and in the case of a positive test for *in vivo* assessment of the effect of the drug on the QT interval in animals prior to its use in humans (<http://www.ich.org>). However, not all drugs with I_{Kr} blocking or QT prolonging actions are proarrhythmic.^{3–5} Widely used drugs such as verapamil, ranolazine, amiodarone and fluoxetine, which block I_{Kr} with or without QT prolongation at their clinically relevant concentrations, are associated with little or no TdP, and in some cases can protect from TdP induced by other agents.⁶ From a mechanistic point of view, other factors, such as dispersion of repolarization, have been shown to contribute to the development of TdP.^{5,7–9}

There are a number of *in vitro* and *in vivo* experimental models available for the preclinical assessment of the TdP potential of new agents. However, few have been validated in a blinded fashion for their positive and negative predictive value.^{2,10} Consequently, the precise sensitivity and specificity of currently available preclinical models remain largely unknown.

Since the arterially perfused ventricular wedge preparation was first developed by Yan and Antzelevitch in 1995,¹¹ this model has been used as a powerful tool for studying the mechanisms underlying arrhythmogenesis associated with a wide variety of sudden cardiac death syndromes.^{7,12–16} As previously reported, the rabbit left ventricular wedge preparation is a sensitive model that evaluates several indices of increased risk for TdP, including QT or action potential prolongation, enhanced transmural dispersion of repolarization (TDR), phase 2 early afterdepolarization (EAD) and EAD-mediated R-on-T extrasystoles.^{2,7} These studies suggest that the isolated arterially-perfused rabbit left ventricular wedge preparation is a potentially promising preclinical model.

The present study provides validation of the model in a randomized, blinded study designed to evaluate thirteen compounds with well established cardiac safety profiles in terms of TdP risk.

Methods

Arterially perfused rabbit left ventricular wedge preparations

The Institutional Animal Care and Use Committee (IACUC) approved the use of animals in this project. Surgical preparation of the rabbit left ventricular wedge has been described in detail in previous publications.⁷ Briefly, female rabbits (New Zealand White) weighing 2.5–3.0 kg were anticoagulated with heparin and anesthetized by intramuscular injection of xylazine (5 mg/kg) and intravenous administration of ketamine · HCl (30–35 mg/kg). The chest was opened via a left thoracotomy, and the heart was excised and placed in a cardioplegic solution consisting of cold (4 °C) normal Tyrode's solution. The left circumflex branch of the coronary artery was cannulated and perfused with the cardioplegic solution. Unperfused areas of the left ventricle, which were easily identified by their reddish appearance due to the

existence of unflushed erythrocytes, were removed. The preparation was then placed in a small tissue bath and arterially perfused with Tyrode's solution containing 4 mM K^+ buffered with 95% O_2 and 5% CO_2 (temperature: $35.7 \pm 0.1^\circ C$, mean perfusion pressure: 35–45 mmHg). The ventricular wedge was allowed to equilibrate in the tissue bath for one hour prior to electrical recordings.

Electrophysiologic recordings from rabbit ventricular wedge preparations

A transmural ECG signal was recorded using extracellular silver/silver chloride electrodes placed in the Tyrode's solution bathing the preparation 1.0 to 1.5 cm from the epicardial and endocardial surfaces, along the same vector as the transmembrane recordings. The QT interval was defined as the time from the onset of the QRS to the point at which the final downslope of the T wave crossed the isoelectric line. Transmembrane action potentials were recorded from epicardium (Epi) and endocardium (Endo) using floating glass microelectrodes. The T_{p-e} interval, which closely approximates transmural dispersion of repolarization (TDR), was defined as the time from the peak to the end of the T wave.

The QT and T_{p-e} intervals were measured manually in three consecutive beats within the last minute of the recording and the values were then averaged.

Study protocols

Thirteen compounds were prepared by the sponsor as DMSO stock solutions (with the exception of sparfloxacin which was dissolved in 0.2 N NaOH) at a concentration of 1000-fold (cisapride, terfenadine, verapamil, thioridazine, clozapine, desipramine) or 300-fold (sparfloxacin, azithromycin, erythromycin, clarithromycin, fexofenadine, captopril, moxifloxacin) greater than the highest concentration administered in this study. The set of 13 compounds was tested in four rounds (total of $4 \times 13 = 52$ rabbits), with the order of testing within each round being assigned by a random number generator. The compound identity, concentration range, molecular weight and order of testing were unknown to the investigators performing the experiments and quantifying the results. After each round, the results were sent to the sponsor prior to initiating the next round of experiments.

Each compound was tested at 4 concentrations such that two concentrations were below and two concentrations were above reported hERG IC_{50} values. After a one hour equilibration period during which the preparation achieved electrical stability,⁷ each preparation was exposed to each of 4 drug concentrations for a period of 20 minutes prior to beginning of data collection. The total drug perfusion time at each concentration was 40 to 50 minutes (Figure 1). Two basic cycle lengths, 1000 ms and 2000 ms, were used to pace the preparation from the endocardial surface.

Our validating experiments for the rabbit left ventricular wedge preparation treated with up to 3/1000 of DMSO ($n = 6$) prior to this blinded study demonstrate that the QT and T_{p-e} intervals change within 2% and 5%, respectively, over a period of 4 hours. Similar data have been obtained in other laboratories.¹⁷

To estimate the relative TdP risk of each compound

The relative TdP risk of each compound was estimated according to the criteria listed in Table 1, modified from those reported previously.^{2,7} The following three core parameters were used in scaling the relative TdP risk of each compound: delayed ventricular repolarization (QT or APD prolongation), dispersion of repolarization (the T_{p-e}/QT ratio or TDR) and the incidence of EAD, with and without closely coupled extrasystoles, and the development of TdP. Among the three parameters, the development of EAD-induced extrasystoles received the greatest weight. The maximum TdP score was 14 and the minimum TdP score was -2.

Compound selection

The compounds selected for this study were chosen on the basis of their known ability to prolong QTc and/or induce TdP in humans (sparfloxacin, moxifloxacin, clarithromycin, erythromycin, azithromycin, cisapride, terfenadine, thioridazine) or their established cardiac safety in clinical usage (verapamil, clozapine, captopril, fexofenadine, desipramine). Multiple agents within a given chemical class were specifically chosen in order to evaluate the ability of the rabbit ventricular wedge model to distinguish pro-arrhythmic liability between structurally similar compounds (two fluoroquinolone antibiotics, three macrolide antibiotics, two antihistamines, two antipsychotics). To ensure that the drug concentration used in the study was correct, effluent was collected from each experiment and sent to the sponsor for analysis. The identity and concentrations of the 13 compounds were disclosed after completion of the study (Table 2).

Statistics

Statistical analysis of the data was performed using Student's t-test for paired and unpaired data between two groups. Chi-square test was used for the comparison between two groups for event incidence. Data are presented as mean $\pm$ SEM; and "n" indicates the number of rabbits.

Results

Representative recordings of rabbit ventricular transmembrane action potentials and ECG in the presence of compounds with different TdP risks are shown in Figure 2. The electrophysiological effects of these four agents (verapamil, azithromycin, terfenadine and cisapride) will be highlighted.

Effects of the compounds on the QT interval

Figure 3 illustrates the concentration-dependent effect of 13 compounds on the QT interval, displayed as a function of multiples of human free therapeutic plasma $C_{\max}$ (TPC). Verapamil, an L-type calcium channel blocker and also a potent *hERG* inhibitor without documented clinical TdP risk, produced negligible QT prolongation at concentrations of 0.01 to 1.0 μ M (Figure 2) and shortened the QT interval at 10 μ M, 125 fold that of its free TPC (Figure 3A). Azithromycin, an antibiotic capable of causing TdP in individuals with reduced ventricular repolarization reserve such as congenital LQTS,¹⁸ produced no significant QT prolongation at doses of 1 μ M and 10 μ M, but prolonged the QT interval by $36.9 \pm 4.8\%$ and $87.1 \pm 4.8\%$ at 100 μ M and 333 μ M, concentrations 100 and 333 times TPC, respectively ($p < 0.01$ vs. control, $n = 4$). Terfenadine, an antihistamine drug with a known TdP risk,¹⁹ produced a concentration-dependent increase in the QT interval at 0.01 to 1 μ M, but shortened it at a higher concentration (10 μ M). The maximal QT prolongation was $21.5 \pm 7.4\%$ at 1 μ M, approximately 50 fold that of free TPC (Figure 3A). Cisapride, a gastrointestinal motility agent with a substantial risk of TdP,²⁰ produced a concentration-dependent QT prolongation between 0.01 and 1 μ M, but attenuated QT prolongation at 10 μ M (Figure 3A). Cisapride significantly prolonged the QT interval at a concentration as low as 2 fold that of TPC. At a concentration of 0.1 μ M, approximately 20 fold that of TPC, cisapride increased the QT interval by $70.0 \pm 16.4\%$ ($p < 0.05$).

Effects of the compounds on the T_{p-e} interval and the T_{p-e}/QT ratio

Figure 3B illustrates the effects of the 13 drugs on the T_{p-e} interval, an index of TDR. Verapamil, a compound negative for TdP, risk, exhibited no significant effect on the T_{p-e} interval at concentrations of 0.01 to 1 μ M, but abbreviated it at 10 μ M ($-22.0 \pm 5.9\%$, $p < 0.05$). Because verapamil abbreviated T_{p-e} interval more than the QT interval at 10 μ M, the T_{p-e}/QT ratio was reduced ($-15.7 \pm 5.9\%$, $p = 0.078$). Azithromycin, a weak QT prolonging agent,

produced no significant T_{p-e} prolongation at concentrations of 1 μM and 10 μM , but increased it at much higher concentrations (100 μM to 333 μM , Figure 3B). Interestingly, QT prolongation in the presence of higher concentrations of azithromycin was accompanied by a proportional increase in the T_{p-e} interval, so that the T_{p-e}/QT ratio remained relatively unchanged (Figure 4). On the other hand, although terfenadine caused only a modest increase in the QT interval (Figures 2 and 3), it produced a more marked increase in the T_{p-e} interval. At a concentration of 1 μM , the concentration at which the maximal QT prolongation occurred, terfenadine prolonged the T_{p-e} interval by $46.8 \pm 12.7\%$ ($p < 0.05$). Terfenadine increased the T_{p-e} interval more than QT interval, leading to a significant increase in the T_{p-e}/QT ratio ($20.3 \pm 2.9\%$ at 1 μM , $p < 0.01$, Figure 4).

Agents that induced EADs, including cisapride, clarithromycin, sparfloxacin and erythromycin, tended to produce a greater prolongation of T_{p-e} interval (Figure 3B) and T_{p-e}/QT interval ratio (Figure 4). At a concentration of 1 μM , cisapride induced EADs and R-on-T extrasystoles in 4 of 4 preparations ($p < 0.01$, Figure 2) and increased the T_{p-e} interval and T_{p-e}/QT ratio by $183.3 \pm 19.1\%$ ($p < 0.01$) and $63.2 \pm 11.5\%$ ($p < 0.05$), respectively (Figures 3B and Figure 4). Figure 5 shows the dynamic interaction and relationship between the appearance of EADs in the endocardium and the T_{p-e} interval in a representative experiment. A further increase in cisapride dose (10 μM) resulted in disappearance of EADs in all of 4 preparations ($p < 0.01$ vs. the incidence at dose of 1 μM). Interestingly, the T_{p-e}/QT ratio decreased markedly to $-8.4 \pm 3.7\%$ ($p < 0.01$ vs. $63.2 \pm 11.5\%$ at dose of 1 μM) despite the fact that both of the QT and T_{p-e} intervals were still significantly prolonged (Figure 3).

Effects of the compounds on EAD-dependent phenomena

Among the thirteen compounds tested, compounds for which there are a number of published case reports for TdP,³ such as erythromycin, sparfloxacin, cisapride and clarithromycin, resulted in appearance of well defined phase 2 EADs. EAD-induced R-on-T extrasystoles capable of precipitating TdP were observed with cisapride and clarithromycin at relatively high concentrations (1 μM and 333 μM , respectively). Figure 2 illustrates an example of the effect of 1 μM cisapride to induce EADs and R-on-T extrasystoles. Although moxifloxacin prolonged the QT interval and APD more dramatically when compared with other QT prolonging agents, it did not produce any EADs at the concentrations tested.

Estimated TdP risk

The relative TdP risk of each compound was estimated according to the criteria defined in Table 1. Points were assigned based on the effect of each compound on the QT interval, T_{p-e}/QT ratio, EAD and EAD-induced triggered activity. At concentrations up to 100 fold that of free TPC, verapamil, clozapine, captopril, fexofenadine and desipramine received a maximal TdP score of equal to or less than 0.25; whereas azithromycin, thioridazine, terfenadine, moxifloxacin, erythromycin, sparfloxacin, cisapride and clarithromycin received TdP scores ranging from 1.00 to 7.25 (Figure 6).

Discussion

Our data provide validation of the arterially perfused rabbit ventricular wedge preparation as a preclinical experimental model for assessment of drugs with TdP liability. The relative risk of 13 drugs with and without known TdP liability was evaluated using a quantitative approach in which all investigators and technicians were blinded to the names, concentrations and molecular weights of the drugs. The results point to the isolated arterially perfused rabbit ventricular wedge preparation as a preclinical model with a high sensitivity and specificity for identifying drugs with a potential for causing TdP.

The incidence of drug-induced TdP ranges from <0.1% for noncardiac agents such as terfenadine or moxifloxacin up to 3–8% for some antiarrhythmic drugs, such as quinidine.^{21–24} Drugs with a relatively low TdP liability may be difficult to identify without a preclinical model of high sensitivity designed to avoid false negatives.^{2,8} Indeed, regulatory agencies have emphasized the importance of a high sensitivity in preclinical testing.²⁵

The sensitivity of a preclinical test is determined, in part, by the species selected. Among those available for electrophysiological study, the rabbit is well-suited for simulating humans with a markedly reduced repolarization reserve due to its intrinsically weak I_{Ks} throughout the ventricular endocardium.²⁶ As a consequence, the entire endocardium in the adult rabbit exhibits characteristics of M cells, which are known to be exquisitely sensitive to QT prolonging drugs, owing in part to the weaker I_{Ks} in this cell type. This characteristic of the rabbit ventricle, particularly in female gender, renders it exceptionally sensitive even to weak QT prolonging agents.^{2,7,8,27} In the present study, a positive signal for QT prolongation was observed for all drugs with a known TdP risk, even for drugs that tested negative in other preclinical models. For example, terfenadine, a non-cardiac drug with a TdP risk in humans, caused little QT or action potential prolongation in conscious dogs,²⁸ or in isolated canine Purkinje fibers,²⁹ porcine myocardium or Purkinje fibers,³⁰ but prolonged QT interval by more than 20% in the rabbit left ventricular wedge preparation and increased dispersion of repolarization by nearly 50%.

Electrophysiological stability of a preclinical model is another important factor that influences the predictive value of preclinical testing. Electrophysiological instability reduces the signal to noise ratio of the test, and may produce false negatives as well as false positives. Because the ventricular wedge preparation is arterially-perfused via the native coronary artery, the preparation remains electrically stable for at least 4 hours.^{17,31,32} In our hands, the QT interval of the rabbit left ventricular wedge preparation changes less than 2% over a period of four hours after the initial one hour equilibration period. Thus, the wedge preparation permits a temporal window sufficient for studying multiple concentrations of a drug, with a long exposure at each level.

Although regulatory agencies have expressed particular interest in the sensitivity of the preclinical test, specificity is of equal importance to the pharmaceutical industry in order to avoid false positives that could unwarrantedly remove promising drugs from their pipeline. While measurements of the QT interval or of hERG inhibition can detect drugs with a TdP liability with great sensitivity, this approach often lacks specificity. hERG inhibition alone in many cases does not translate directly into TdP risk, since drugs like verapamil, ranolazine, sodium pentobarbital and fluoxetine, possess hERG-blocking activities at clinically relevant concentrations, but are not associated with TdP.^{3,4} Agents like amiodarone, which also blocks I_{Kr} produce marked QT prolongation but rarely cause TdP even in patients who have previously developed TdP as a complication of other QT prolonging agents.³³

The T_{p-e}/QT ratio and the incidence of EADs and EAD-induced triggered activity are additional parameters used in the present study to assess the relative risk for drug-induced TdP. These parameters increase both the sensitivity and specificity of the rabbit ventricular wedge preparation because they provide unique signals specifically related to the development of TdP.^{7–9} Spatial dispersion of repolarization and EAD-induced triggered activity are well recognized as the substrate and trigger for the development of TdP.⁹ Interestingly, the T_{p-e}/QT ratio may serve as an important parameter useful not only in differentiating between potent and weak I_{Kr} blockers but also in distinguishing pure I_{Kr} blockade from combined inhibition of I_{Kr} and inward currents. Compounds with a measurable incidence of TdP risk such as cisapride, clarithromycin, sparfloxacin, erythromycin, dofetilide and sotalol amplify transmural dispersion of repolarization by preferentially prolonging sub- and endocardial

action potential duration due to their effect on I_{Kr} and therefore markedly increase the T_{p-e}/QT ratio (Figures 2, 4 and 5).^{2,7} Some agents such as terfenadine at lower dose cause only a modest increase of QT interval, but a significant increase in the T_{p-e}/QT ratio ($20.3 \pm 2.9\%$), identifying it as a drug with significant TdP liability. It is noteworthy that previous preclinical models have failed to identify terfenadine as having a TdP risk. On the other hand, although azithromycin significantly prolongs both of the QT and T_{p-e} intervals at higher doses, the T_{p-e}/QT ratio remains relatively unchanged probably due to the fact that azithromycin inhibits inward sodium current at the same dose range (our unpublished data). This is probably one of the reasons why azithromycin carries a much smaller TdP risk than erythromycin clarithromycin and sparfloxacin. Similarly, cisapride at higher doses, which inhibits inward currents,³⁴ markedly reduces the T_{p-e}/QT ratio, and abolishes EADs and R-on-T ectopic beats despite the fact both the QT and T_{p-e} intervals remain prolonged.

The safety margin, clinical pharmacokinetic profile and pharmacodynamics of each compound must all be taken into consideration in the final analysis. For example, fexofenadine causes a significant increase in the QT and T_{p-e} intervals and received a significant TdP score at doses greater than 100 fold its free TPC (Figures 3 and 6). This is consistent with its clinical outcome, which is that the drug has a very low risk of TdP.³⁵ Moxifloxacin, a fluoroquinolone antibiotic that modestly prolongs the QT interval in humans, received a small but significant TdP score at concentrations ranging from 5 to 50 fold its free TPC (Figure 6). These results are consistent with clinical outcome data demonstrating a low incidence of TdP.

The isolated Langendorff-perfused rabbit heart preparation as a preclinical model to identify drugs with TdP liability was recently validated in blinded assessments, and it exhibited a high sensitivity.^{36,37} The isolated arterially-perfused rabbit ventricular wedge preparation differs from the Langendorff-perfused rabbit heart preparation with regard to two critically important aspects. First, the rabbit ventricular wedge preparation is electrically stable for more than 4 hours, as demonstrated by us and others.¹⁷ In contrast, the Langendorff-perfused rabbit heart preparation deteriorates faster, showing QT interval abbreviation of $>3.5\%$ (11 ms) over a one hour perfusion period.^{36,38} Drug testing in the latter must therefore be completed within one to two hours, necessitating relatively short (10 min) exposure to each drug concentration.^{36,37} This is an important limitation in the case of drugs that have a delayed time-course of action due to slow intracellular accumulation.³⁹ Secondly, studies involving the wedge preparation permit measurement of three core parameters specifically related to the development of TdP: the QT interval or APD, TDR or T_{p-e} and EAD-induced triggered activity, in addition to TdP. Relative TdP risk can therefore be scored semi-quantitatively. Based on preclinical tests involving measurement of monophasic action potentials in the Landendorff preparation, Hondeghem et al. introduced the interesting concept of TRIaD (e.g. triangulation, reverse use dependence and instability) for the assessment of drug-induced TdP risk, which still remains to be fully validated. Interpretation of the data is difficult at times. For example, amiodarone, a QT prolonging agent, which rarely causes TdP in humans, produces triangulation, reverse use dependence and instability.³⁷

In summary, our blinded study validates the isolated arterially perfused rabbit ventricular wedge preparation as a promising preclinical model for the assessment of drug-induced proarrhythmia. This approach can not only distinguish between TdP positive and negative agents but can also scale the relative risk of each compound from integrated information of QT prolongation, transmural dispersion and EAD activity.

Limitations of the study

Analysis of the effluent collected from each preparation yielded drug concentrations consistent with the prepared stock solution with one exception. The concentrations of thioridazine were significantly lower than that in the corresponding stock solutions, suggesting the possibility

that the compound might have been partially adsorbed to glassware and the perfusion tubing. This may be the reason why thioridazine, which is associated with a fairly high TdP risk,³ received TdP score that may potentially underestimate its potential risk.

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References

1. Selzer A, Wray HW. Quinidine syncope. Paroxysmal ventricular fibrillation occurring during treatment of chronic atrial arrhythmias. *Circulation* 1964;30:17–26. [PubMed: 14197832]
2. Joshi A, DiMino T, Vohra Y, Cui C, Yan GX. Preclinical strategies to assess QT liability and torsadogenic potential of new drugs: the role of experimental models. *J Electrocardiol* 2004;37(Suppl): 7–14. [PubMed: 15534787]
3. Redfern WS, Carlsson L, Davis AS, Lynch WG, MacKenzie I, Palethorpe S, Siegl PK, Strang I, Sullivan AT, Wallis R, Camm AJ, Hammond TG. Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. *Cardiovasc Res* 2003;58:32–45. [PubMed: 12667944]
4. Milberg P, Ramtin S, Monnig G, Osada N, Wasmer K, Breithardt G, Haverkamp W, Eckardt L. Comparison of the in vitro electrophysiologic and proarrhythmic effects of amiodarone and sotalol in a rabbit model of acute atrioventricular block. *J Cardiovasc Pharmacol* 2004;44:278–286. [PubMed: 15475823]
5. Antzelevitch C. Arrhythmogenic mechanisms of QT prolonging drugs: is QT prolongation really the problem? *J Electrocardiol* 2004;37(Suppl):15–24. [PubMed: 15534788]
6. Shimizu W, Ohe T, Kurita T, Kawade M, Arakaki Y, Aihara N, Kamakura S, Kamiya T, Shimomura K. Effects of verapamil and propranolol on early after-depolarizations and ventricular arrhythmias induced by epinephrine in congenital long QT syndrome. *J Am Coll Cardiol* 1995;26:1299–1309. [PubMed: 7594047]
7. Yan GX, Wu Y, Liu T, Wang J, Marinchak RA, Kowey PR. Phase 2 early afterdepolarization as a trigger of polymorphic ventricular tachycardia in acquired long-QT syndrome: Direct evidence from intracellular recordings in the intact left ventricular wall. *Circulation* 2001;103:2851–2856. [PubMed: 11401944]
8. Lankipalli RS, Zhu T, Guo D, Yan GX. Mechanisms underlying arrhythmogenesis in long QT syndrome. *J Electrocardiol* 2005;38(Suppl):69–3. [PubMed: 16226078]
9. Belardinelli L, Antzelevitch C, Vos MA. Assessing predictors of drug-induced torsade de pointes. *Trends Pharmacol Sci* 2003;24:619–625. [PubMed: 14654302]
10. Lawrence CL, Pollard CE, Hammond TG, Valentin JP. Nonclinical proarrhythmia models: predicting Torsades de Pointes. *J Pharmacol Toxicol Methods* 2005;52:46–59. [PubMed: 15975832]
11. Yan GX, Antzelevitch C. Cellular basis for the electrocardiographic J wave. *Circulation* 1996;93:372–379. [PubMed: 8548912]
12. Yan GX, Shimizu W, Antzelevitch C. The characteristics and distribution of M cells in arterially-perfused canine left ventricular wedge preparations. *Circulation* 1998;98:1921–1927. [PubMed: 9799214]
13. Yan GX, Antzelevitch C. Cellular basis for the Brugada syndrome and other mechanisms of arrhythmogenesis associated with ST-segment elevation. *Circulation* 1999;100:1660–1666. [PubMed: 10517739]
14. Yan GX, Joshi A, Guo D, Hlaing T, Martin J, Xu X, Kowey PR. Phase 2 reentry as a trigger to initiate ventricular fibrillation during early acute myocardial ischemia. *Circulation* 2004;110:1036–1041. [PubMed: 15302777]

15. Shimizu W, Antzelevitch C. Differential effects of beta-adrenergic agonists and antagonists in LQT1, LQT2 and LQT3 models of the long QT syndrome. *J Am Coll Cardiol* 2000;35:778–786. [PubMed: 10716483]
16. Nam GB, Burashnikov A, Antzelevitch C. Cellular mechanisms underlying the development of catecholaminergic ventricular tachycardia. *Circulation* 2005;111:2727–2733. [PubMed: 15911700]
17. Chen X, Cass JD, Bradley JA, Sun Z, Zhou J. Use of arterially perfused rabbit ventricular wedge in predicting arrhythmogenic potentials of drugs. *J Pharmacol Toxicol Methods*. 2006in press
18. Arellano-Rodrigo E, Garcia A, Mont L, Roque M. Torsade de pointes and cardiorespiratory arrest induced by azithromycin in a patient with congenital long QT syndrome. *Med Clin (Barc)* 2001;117:118–119. [PubMed: 11459586]
19. Slater JW, Zechin AD, Haxby DG. Second-generation antihistamines: a comparative review. *Drugs* 1999;57:31–47. [PubMed: 9951950]
20. Doig JC. Drug-induced cardiac arrhythmias: incidence, prevention and management. *Drug Saf* 1997;17:265–275. [PubMed: 9352962]
21. Shah RR. Drug-induced prolongation of the QT interval: regulatory dilemmas and implications for approval and labelling of a new chemical entity. *Fundam Clin Pharmacol* 2002;16:147–156. [PubMed: 12031067]
22. Moller M, Torp-Pedersen CT, Kober L. Dofetilide in patients with congestive heart failure and left ventricular dysfunction: safety aspects and effect on atrial fibrillation. The Danish Investigators of Arrhythmia and Mortality on Dofetilide (DIAMOND) Study Group. *Congest Heart Fail* 2001;7:146–150. [PubMed: 11828153]
23. Lehmann MH, Hardy S, Archibald D, Quart B, Macneil DJ. Sex difference in risk of torsade de pointes with d,l-sotalol. *Circulation* 1996;94:2535–2541. [PubMed: 8921798]
24. Bauman JL, Bauernfeind RA, Hoff JV, Strasberg B, Swiryn S, Rosen KM. Torsade de pointes due to quinidine: Observations in 31 patients. *Am Heart J* 1984;107:425–430. [PubMed: 6695683]
25. Caverio I, Crumb W. Moving towards better predictors of drug-induced Torsade de Pointes. *Expert Opin Drug Saf* 2006;5in press
26. Xu X, Rials SJ, Wu Y, Salata JJ, Liu T, Bharucha D, Marinchak RA, Kowey PR. Left ventricular hypertrophy decreases slowly, but not rapidly activating delayed rectifier K⁺ currents of epicardial and endocardial myocytes in rabbits. *Circulation* 2001;103:1585–1590. [PubMed: 11257089]
27. Lu HR, Remeysen P, Somers K, Saels A, De Clerck F. Female gender is a risk factor for drug-induced long QT and cardiac arrhythmias in an in vivo rabbit model. *J Cardiovasc Electrophysiol* 2001;12:538–545. [PubMed: 11386514]
28. Fossa AA, Depasquale MJ, Raunig DL, Avery MJ, Leishman DJ. The relationship of clinical QT prolongation to outcome in the conscious dog using a beat-to-beat QT-RR interval assessment. *J Pharmacol Exp Ther* 2002;302:828–833. [PubMed: 12130750]
29. Champeroux P, Viaud K, El Amrani AI, Fowler JS, Martel E, Le Guennec JY, Richard S. Prediction of the risk of Torsade de Pointes using the model of isolated canine Purkinje fibres. *Br J Pharmacol* 2005;144:376–385. [PubMed: 15655517]
30. Gintant GA, Limberis JT, McDermott JS, Wegner CD, Cox BF. The canine Purkinje fiber: an in vitro model system for acquired long QT syndrome and drug-induced arrhythmogenesis. *J Cardiovasc Pharmacol* 2001;37:607–618. [PubMed: 11336111]
31. Antzelevitch C, Sun ZQ, Zhang ZQ, Yan GX. Cellular and ionic mechanisms underlying erythromycin-induced long QT and torsade de pointes. *J Am Coll Cardiol* 1996;28:1836–1848. [PubMed: 8962574]
32. Yan GX, Antzelevitch C. Cellular basis for the normal T wave and the electrocardiographic manifestations of the long-QT syndrome. *Circulation* 1998;98:1928–1936. [PubMed: 9799215]
33. Mattioni TA, Zheutlin TA, Sarmiento JJ, Parker M, Lesch M, Kehoe RF. Amiodarone in patients with previous drug-mediated torsade de pointes. Long-term safety and efficacy. *Ann Intern Med* 1989;111:574–580. [PubMed: 2774388]
34. Di Diego JM, Belardinelli L, Antzelevitch C. Cisapride-induced transmural dispersion of repolarization and torsade de pointes in the canine left ventricular wedge preparation during epicardial stimulation. *Circulation* 2003;108:1027–1033. [PubMed: 12912819]

35. Dhar S, Hazra PK, Malakar S, Mistri G. Fexofenadine-induced QT prolongation: a myth or fact? *Br J Dermatol* 2000;142:1260–1261. [PubMed: 10848773]
36. Hondeghem LM, Hoffmann P. Blinded test in isolated female rabbit heart reliably identifies action potential duration prolongation and proarrhythmic drugs: importance of triangulation, reverse use dependence, and instability. *J Cardiovasc Pharmacol* 2003;41:14–24. [PubMed: 12500017]
37. Hondeghem LM, Lu HR, van Rossem K, De Clerck F. Detection of proarrhythmia in the female rabbit heart: blinded validation. *J Cardiovasc Electrophysiol* 2003;14:287–294. [PubMed: 12716112]
38. Hondeghem LM, Carlsson L, Duker G. Instability and triangulation of the action potential predict serious proarrhythmia, but action potential duration prolongation is antiarrhythmic. *Circulation* 2001;103:2004–2013. [PubMed: 11306531]
39. Antzelevitch, C.; Davidenko, JM.; Sicouri, S., et al. Electrophysiologic effects of quinidine in canine Purkinje fibers and ventricular myocardium. Slow development of the antiarrhythmic and arrhythmogenic effects of the drug. In: Velasco, M.; Israel, A.; Romero, E.; Silva, H., editors. *Recent Advances in Pharmacology and Therapeutics*. New York: Ecerpta Medica; 1989. p. 259-263.
40. Clifford CP, Adams DA, Murray S, Taylor GW, Wilkins MR, Boobis AR, Davies DS. The cardiac effects of terfenadine after inhibition of its metabolism by grapefruit juice. *Eur J Clin Pharmacol* 1997;52:311–315. [PubMed: 9248771]
41. Kang J, Wang L, Chen XL, Triggie DJ, Rampe D. Interactions of a series of fluoroquinolone antibacterial drugs with the human cardiac K⁺ channel HERG. *Molec Pharmacol* 2001;59:122–126. [PubMed: 11125032]
42. Klasco, RK., editor. *Physician's Desk Reference Online*. Colorado: Thomson Micromedex; 2005. Drugdex Drug Evaluation.
43. Stanat SJ, Carlton CG, Crumb WJ Jr, Agrawal KC, Clarkson CW. Characterization of the inhibitory effects of erythromycin and clarithromycin on the HERG potassium channel. *Mol Cell Biochem* 2003;254:1–7. [PubMed: 14674677]
44. Lacerda AE, Kramer J, Shen KZ, Thomas D, Brown AM. Comparison of block among cloned cardiac potassium channels by non-antiarrhythmic drugs. *Eur Heart J Supplements* 2001;3(Suppl K):K23–K30.
45. Kongsamut S, Kang J, Chen XL, Roehr J, Rampe D. A comparison of the receptor binding and HERG channel affinities for a series of antipsychotic drugs. *Eur J Pharmacol* 2002;450:37–41. [PubMed: 12176106]
46. Zhang S, Zhou Z, Gong Q, Makielski JC, January CT. Mechanism of block and identification of the verapamil binding domain to HERG potassium channels. *Circ Res* 1999;84:989–998. [PubMed: 10325236]
47. Warner B, Hoffmann P. Investigation of the potential of clozapine to cause torsade de pointes. *Adverse Drug React Toxicol Rev* 2002;21:189–203. [PubMed: 12503253]

Figure 1.

The drug testing protocol used. “R” denotes time of electrophysiological recording. “1000 ms” and “2000 ms” represent BCLs of 1000 ms and 2000 ms, respectively.

Figure 2.

Representative original recordings of transmembrane action potentials and ECGs from the rabbit left ventricular wedge preparation in the presence of verapamil, azithromycin, terfenadine and cisapride, which represent different levels of TdP risk. The T_{p-e} interval approximated closely transmural dispersion of repolarization, marked by the dotted lines. BCL = 2000 ms.

Figure 3.

Comparison of the dose-dependent effect on the QT (A) and T_{p-e} (B) intervals. BCL = 2000 ms. The free TPC of the compounds was obtained from previously published studies.^{3,40–42} The symbols of * and ** indicate $p < 0.05$ and $p < 0.01$, respectively, when compared with the control value of the compound. $n = 4$ for each compound.

Figure 4.

Maximal drug-induced change in T_{p-e}/QT ratio at concentrations <100 fold of their free TPC. The black bars represent the compounds that resulted in significant QT prolongation accompanied by the development of EAD, R-on-T extrasystoles and TdP; the gray bars represent the compounds that led to a significant increase in the QT interval but without EAD and EAD-dependent phenomena; and the blank bars represent the negative compounds. The symbol of * indicates $p < 0.05$ when compared with the control value of the compound. $n = 4$ for each compound, and BCL = 2000 ms.

Figure 5.

A. Cisapride ($1 \mu\text{M}$)-induced phase 2 EADs and increased T_{p-e} interval in the rabbit ventricular wedge preparation. Phase 2 EADs appeared after pacing cycle length was increased from 1000 ms to 2000 ms. Each panel shows transmembrane action potentials from the endocardium and epicardium and an ECG. The last grouping illustrates an example of EAD-induced triggered extrasystole. The break symbol (//) between beats indicates that these ventricular beats are not consecutive and selected for better illustration of a dynamic change in EADs. **B.** Dynamic changes in the QT and T_{p-e} intervals plotted as a function of consecutive beat number after an increase in pacing cycle length from 1000 ms to 2000 ms during which the size of phase 2 EAD was increased gradually. “EAD” indicates that EAD was seen from that beat. **C.** Dynamic change in the T_{p-e}/QT ratio plotted as a function of beat number. “EAD” indicates that EAD was seen from that beat.

Figure 6.

TdP scores derived based on the criteria in Table 1 BCL = 2000 ms. The free TPC of the compounds was obtained from previously published studies.^{3,40-42} The symbols of * and ** indicate $p < 0.05$ and $p < 0.01$, respectively, when compared with the control value of the compound. $n = 4$ for each compound.

Score system for quantitating relative TdP risk compound. Each compound is evaluated changes in QT interval, T_{p-e}/QT ratio and development of phase 2 early afterdepolarizations (EAD) with and without a closely coupled extrasystole

	-1	0	1	2	3
Δ QT interval (%)	< -5%	-5% -< 10%	10% -< 20%	20% -< 30%	> 30%
Δ T _{p-e} /QT ratio (%)	< -5%	> -5% -< 10%	10% -< 20%	20% -< 30%	> 30%
Phase 2 EAD		2 ±EAD *	4 EAD without "R-on-T" extrasystole	6 EAD with "R-on-T" extrasystole	8 TdP

BCL = 2000 ms;
* : " ± EAD" indicates appearance of equivocal EAD at a BCL of 2000 ms that became more obvious at a BCL of 4000 ms; the maximal TdP score is 14.

Table 2
Names and doses of 13 compounds tested, and their published hERG IC₅₀ and free human TPC

	Concentrations Tested (μM)				hERG IC ₅₀ (μM)	free TPC (μM)
	Dose 1	Dose 2	Dose 3	Dose 4		
erythromycin	1	10	100	333	39 ⁴³	10 ³
sparfloxacin	1	10	100	333	18 ⁴¹	1.8 ⁴¹
cisapride	0.01	0.1	1	10	0.04 ⁴⁴	0.005 ³
moxifloxacin *	3	30	300	1000	129 ⁴¹	5.9 ⁴¹
Terfenadine	0.01	0.1	1	10	0.056 ⁴⁴	0.017 ⁴⁰
clarithromycin	1	10	100	333	46 ⁴³	1.25 ³
azithromycin	1	10	100	333	NA	1 ⁴²
thioridazine	0.01	0.1	1	10	0.2 ⁴⁵	1 ³
fexofenadine	1	10	100	333	13 ⁴⁴	0.4 ³
verapamil	0.01	0.1	1	10	0.14 ⁴⁶	0.08 ³
clozapine	0.01	0.1	1	10	0.2 ⁴⁷	0.04 ⁴²
captopril	1	10	100	333	NA	1.7 ⁴²
desipramine	0.1	1	10	30	1 ³	0.05 ⁴²

NA: not available;

* : in the presence of P450 inhibition.