Pre-Clinical Assessment of Drug-Induced QT Interval Prolongation. Current Issues and Impact on Drug Discovery

Bernard Fermini and Anthony A. Fossa

Pfizer Global Research and Development, Eastern Point Road, Groton, CT 06340, USA

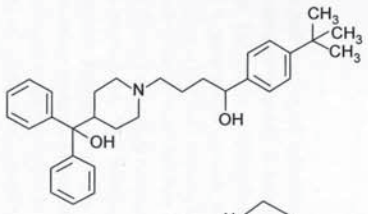
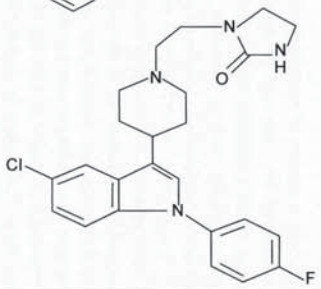
Contents

1. INTRODUCTION

Over the past decade, a number of non-cardiovascular drugs have had their label revised, or have been withdrawn from the market, because of unexpected post-marketing reports of sudden cardiac death associated with a prolongation of the QT interval on surface electrocardiograms (ECG), and an increased risk of developing a rare polymorphic ventricular tachyarrhythmia called torsades de pointes (TdP) (Table 1). Although a direct link between prolongation of the QT interval and the onset of arrhythmias remains to be demonstrated with certainty, it is currently assumed that even a small increase in the QT interval is associated with some risk of developing TdP. As a result, the issue of drug-induced QT prolongation has become the subject of intense regulatory review. QT prolongation is now the leading cause for the withdrawal of approved drugs from the market, and represents a major hurdle for the development process of a myriad of potentially new drugs. Consequently, it has become imperative for the pharmaceutical industry to implement strategies to address this critical issue. Various assays and models are being considered by both regulators and the drug industry to identify, as early as possible in the discovery process, new chemical entities that have the propensity to affect the QT interval in an effort to avoid future health concerns for patients, as well as commercial fallout from adverse labeling and costly withdrawals from the market place.

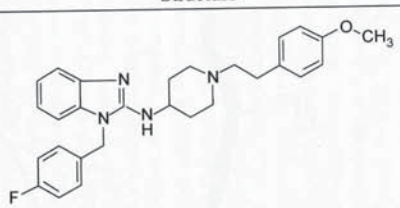
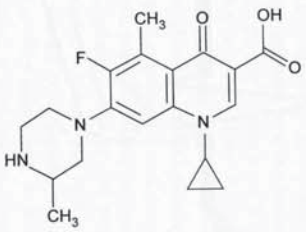
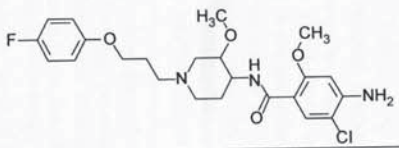
ANNUAL REPORTS IN MEDICINAL CHEMISTRY, VOLUME 39
ISSN: 0065-7743 DOI 10.1016/S0065-7743(04)39024-X

Table 1. Drugs withdrawn for the US market

Drug	Structure	Class	HERG IC_{50} (nM)	Date withdrawn
Terfenadine		Antihistamine	204	Feb 1998
Sertindole		Antipsychotic	14	Dec 1998

(Continued)

Table 1. Continued

Drug	Structure	Class	HERG IC_{50} (nM)	Date withdrawn
Astemizole		Antihistamine	0.9	June 1999
Grepafloxacin		Antibiotic	50,000	Nov 1999
Cisapride		GI prokinetic	6.5	July 2000

This chapter addresses the issue of drug-induced QT prolongation, and examines its impact on drug-development programs in the pharmaceutical industry.

2. ELECTROPHYSIOLOGICAL MECHANISMS UNDERLYING QT PROLONGATION

The surface ECG is a reflection of the sum of action potentials generated from all the cells in the heart. Action potentials result from the opening and closing of membrane-spanning proteins that form ion channels in the membrane. A sequential change in the inward and outward flow of positive ions through these selective channels determines the complex morphology and duration of cardiac action potentials (Fig. 1). Rapid entry of sodium ions initiates the depolarization of the ventricles (phase 0), followed by a rapid repolarization though transiently activating and inactivating outward potassium channels (phase 1). This is followed by a plateau phase (phase 2), mainly determined by the entry of calcium ions through L-type calcium channels, and a repolarization (phase 3) phase resulting from the inactivation of calcium channels, and the increase in net outward potassium currents carried mainly by the slow (I_{Ks}) and rapid (I_{Kr}) components of the delayed rectifier potassium channels. In humans, I_{Kr} appears to play a significant role in determining ventricular action potential duration (APD) and repolarization, as congenital mutations of this channel are associated with a decrease in current amplitude and prolongation the QT interval [1]. Clinically, cardiac repolarization is assessed by measuring the QT interval on

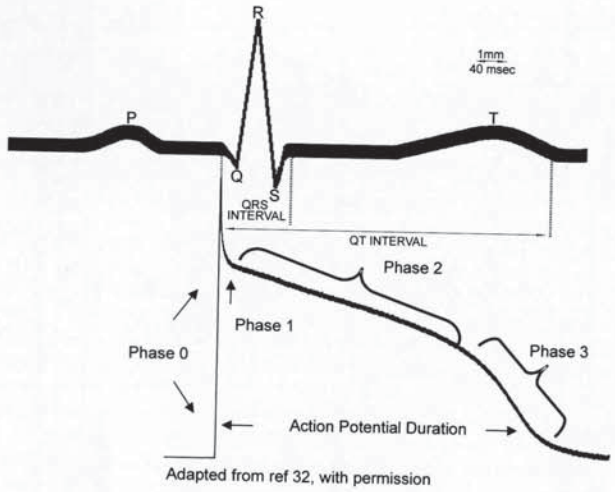


Fig. 1.

the ECG, which is defined as the duration between the beginning of the QRS complex and the end of the T wave, and is a reflection of ventricular APD (Fig. 1). When corrected for individual heart rate, the QT interval is defined as 'corrected' QT, or QTc. Normal upper limit for QTc is approximately 430 ms for men, and 450 ms for women. Drugs that prolong the APD or the QT interval may do so by interacting with one or several ion channels. Excessive APD prolongation (typically in Purkinje fibers and/or midmyocardial cells) increases the propensity of developing spontaneous oscillations of the membrane potential that can give rise to one or more premature responses called early after-depolarizations (EADs). When generated in the presence of transmural heterogeneity in ventricular repolarization, EADs are believed to contribute to the generation of extrasystoles that can trigger TdP [2].

3. QT PROLONGATION AND TORSADES DE POINTES

Prolongation of the QT interval in patients is usually observed in response to an underlying medical condition, or in association with drug treatment, or else in patients afflicted with one of the congenital long-QT syndromes. Genetic studies have identified at least six genes that, if mutated, result in ion channel malfunction that can cause the long-QT syndrome. Studies of one of these genes (KCNH2), the human ether-à-go-go-related gene (HERG at the LQT2 locus), which encodes the cardiac potassium channel I_{Kr}, has provided invaluable information in understanding the mechanisms underlying drug-induced QT prolongation [3,4]. In the large majority of cases, drugs that prolong the QT interval and cause TdP inhibit HERG or I_{Kr} at therapeutic, or supra therapeutic concentrations. Wide ranges of chemical structures developed against many different molecular targets have been shown to inhibit the HERG channel, relative to other voltage-dependent K^+ channels. It is believed that most of the drugs that inhibit HERG current bind to a site located in the intracellular region of the pore cavity of the channel. Recent studies have revealed that the pore of HERG channels is large, which makes it more likely to trap small molecules of different classes, or compounds that are too large to block other potassium channels [5]. Moreover, unlike other potassium channels, HERG channels inactivate rapidly, effectively trapping drug molecules in the vestibule of the channel, leading to an increase in drug concentration near the pore [6]. Using homology modeling of the HERG channel, based on the crystallographic structure of two bacterial potassium channels (KcsA and MthK) representing the closed and open states of the channels, investigators were able to show that the HERG channel also contains two aromatic residues in each S6 helix (Tyr652 and Phe656), which undoubtedly contributes to allowing many different chemical structures to dock and bind to the inner mouth of the channel [5,7]. Such residues are not found in other potassium channels that are less susceptible to drug inhibition. More recently, ligand-based in silico models of the HERG channel have been published, and should help us further our understanding of how drug molecules interact with this channel [8,9].

Although prolongation of the QT interval can lead to life-threatening arrhythmias, in reality the incidence of such arrhythmias is rare. For example, the antibiotic agent grepafloxacin was removed from the US market in November 1999. Out of over

2.7 million prescriptions filled for this drug, there were seven cardiovascular events related to fatalities, with three documented cases of TdP [10]. Currently, more than fifty drugs have been reported to prolong the QT interval and/or induce TdP (see http://www.arizonacert.org). Among the most common are the three antiarrhythmic agents, Ibutilide (rate of TdP: 2–6%), sotalol (rate of 1.8–4.8%) and quinidine (rate 2–8.8%), as well as the prokinetic agent, cisapride (rate of 1 per 120,000 patients) and the antihistamine, terfenadine (rate of 8%). Of these, grepafloxacin, terfenadine and cisapride have been removed from the US market, as well as others such as astemizole (antihistamine) and sertindole (antipsychotic). All of these drugs have been reported to inhibit HERG current amplitude [11–15]. Hence, early identification of compounds that have the propensity to become pro-arrhythmic poses a challenge to physicians, the pharmaceutical industry and regulators. Taking into account the tens of millions of patients exposed these drugs; it remains that no more than a few dozen cases of TdP have been reported. This raises two important issues: (1) is QT prolongation a valid predictor of TdP and (2) why do only a small proportion of individuals experience TdP while the majority appears unaffected by drugs that produce QT interval prolongation?

3.1. QT prolongation as a surrogate marker for TdP

Clinicians and regulators use the QT interval as a surrogate marker for the prediction of adverse effects such as TdP. Rightfully or not, it is currently assumed that even small changes in the QT interval indicate some risk of TdP, and there is presently no well-established threshold below which a prolonged QT interval is believed to be harmless. Yet, not all drugs that prolong the QT interval cause TdP. For example, the calcium channel blocker verapamil, used for the treatment of hypertension, has been shown to prolong the QT interval in a manner that is correlated to its plasma concentration [16], but there are few described cases of verapamil-induced TdP [17]. Also, the antiarrhythmic agent, amiodarone, consistently prolongs the QT interval to more than 500 ms, but rarely causes TdP [18]. Because both verapamil and amiodarone inhibit ion channels other than I_{Kr}, it has been proposed that such mechanisms may preclude the generation of arrhythmias by either mitigating the prolongation of the action potential expected for I_{Kr} inhibition, or reducing the risk of developing EADs by affecting L-type calcium channels and/or the regulation of intracellular calcium levels. Nonetheless, the overall risk of arrhythmia does appear to increase with increasing QTc. Data on QTc intervals in case reports of TdP on a number of cardiac and non cardiac drugs indicate that a QTc interval of >500 ms was most commonly observed before the TdP event. In support of this argument, recent studies looking at the risk stratification of patients with the congenital Long-QT syndrome, LQT2, indicate that the risk of developing a cardiac event before the age of 40 years increases among patients with QTc intervals over 500 ms [19,20]. Finally, available data suggest that in individual subjects, an increase of 60 ms in peak or maximum QTc interval over baseline is also predictive of a potential risk [21]. Therefore, although the relationship between QT interval prolongation and TdP is imperfect, it will continue to be used as a surrogate marker, until a better clinical alternative is identified.

3.2. Risk factors for drug-induced arrhythmias

Many different factors that influence cardiac repolarization and the duration of the QT interval have been associated with an increased risk of drug-induced arrhythmias. These include age (very young and elderly), gender (female > male), heart rate (bradycardia), cardiac disorders, electrolyte imbalance, disease states (hepatic and renal), and concomitant medication. When present, such factors may contribute to exacerbate the actions of a drug that prolongs the QT interval, and predispose to an increase risk of developing TdP. In support of this idea of individual predisposition, a study of patients prescribed a Class III antiarrhythmic agent showed that those who developed TdP had more drug-induced QT prolongation than those who did not [22]. Moreover, the degree of QTc prolongation in the group with TdP was unrelated to drug dosage, suggesting that those who developed TdP showed an abnormal response to the drug. In some patients, the susceptibility has a genetic basis. Current data suggests that as many as 10–15 percent of the individuals that experience drug-induced TdP may carry mutations associated with the long QT-syndrome that may compromise cardiac repolarization, and aggravate the effects of a drug that is otherwise safe in the absence of such background factors [23]. These data suggest that the simultaneous combination of risk factors and genetics may be required to confer increased risk of drug-induced TdP in a certain population of patients. It is hoped that in the future, genetic research will play an important role in identifying populations at higher risk, so they can be treated accordingly when requiring therapies leading to an increased risk of developing TdP.

4. REGULATORY PERSPECTIVES

In response to increased reports of post-marketing QT prolongation and cardiac adverse events by non-cardiovascular drugs, the Committee for Proprietary Medicinal Products (CPMP) in 1997 issued a 'Points to Consider' document entitled 'The assessment of the potential for QT interval prolongation by non-cardiovascular medicinal products' [24]. This document outlined a series of experimental non-clinical and clinical models for assessing the potential for QT prolongation by non-cardiovascular agents. The non-clinical approaches emphasized *in vitro* electrophysiological studies examining action potentials in isolated cardiac tissues like Purkinje fibers and papillary muscle, whereas the *in vivo* approach focused on large animal assessment effects on blood pressure, heart rate, and more robust measurements of electrocardiogram intervals. The clinical guidance also focused on more intense assessments of electrocardiogram intervals obtained from a larger patient population earlier in development. Specific methods and magnitude of changes were noted in the document, thus opening the debate on the scientific basis and relevance of all these recommendations.

Given the tremendous economic and medical consequence of this issue, the International Conference on Harmonization (ICH) recently established an expert working group (EWG) to draft guidance recommending the incorporation of pre-clinical models predictive of QT prolongation and proarrhythmia in the development process of new drugs [25]. The resultant draft guidance document entitled 'Safety Pharmacology Studies For Assessing The Potential For Delayed Ventricular Repolarization

(QT Interval Prolongation) By Human Pharmaceuticals S7B' was signed as a Step 2 draft document in February 2002 (see http://www.ich.org). At Step 2, a consensus draft document is transmitted by the ICH to the regulatory authorities of the three ICH regions (EU, Japan, USA), for internal and external consultation. The S7B document advocates a testing strategy that incorporates (1) an assay evaluating the block of repolarizing currents such as HERG or I_{Kr}; (2) a repolarization assay that evaluates changes in APD in an integrated cardiac tissue preparation such as Purkinje fibers; and (3) an assay evaluating changes in the QT interval in an *in vivo* preparation. The implication of such guidelines is that any drug that blocks repolarizing currents and prolongs the cardiac APD in the *in vitro* models or prolongs the QT interval or elicits arrhythmias in the *in vivo* model is considered to pose a risk to human. Yet, the absence of findings in these assays is not considered to preclude a potential risk to humans. The Food and Drug Administration (FDA), as an organization, has not issued a written statement or documents on QT prolongation. However, it is supporting a study by the International Life Sciences Institute and Health and Environmental Sciences Institute (ILSI/HESI), evaluating the sensitivity and specificity of the *in vitro* and *in vivo* assays proposed by the ICH S7B. It is also collaborating with Georgetown University in evaluating several drugs known to cause TdP in the HERG assay. Results from this initiative have not yet been published.

5. PRE-CLINICAL MODELS TO ASSESS THE PRO-ARRHYTHMIC POTENTIAL OF NON-CARDIOVASCULAR DRUGS

There has been much debate within the pharmaceutical industry as to the most predictive *in vitro* and *in vivo* models for predicting the risk of TdP in clinical use. A survey of the current practice in the pharmaceutical industry for assessing the potential of QT prolongation by non-cardiovascular drugs showed that there was a wide diversity in the testing methodologies used [26]. A more recent study examined the relative value of pre-clinical cardiac electrophysiology data for predicting the risk of TdP in clinical use [27]. This study also introduced the notion of a safety margin between the IC_{50} for inhibition of the HERG channel, and the maximal effective therapeutic plasma concentration of a drug attained during clinical use. Finally, it proposed a pre-clinical screening scheme with the purpose of acquiring important information on any new compound before it enters clinical evaluation. These assays are similar to that described in the S7B document, and their utility, advantages and disadvantages are now discussed.

5.1. *In vitro* assays

As mentioned previously, two functional assays are regularly used to evaluate the potential of a drug to delay cardiac repolarization. One assay looks at the effects of drugs on the action potential of cardiac tissues obtained from the myocardium of animal species demonstrating an ionic profile similar to that of the human heart (including dog, rabbit, ferret, swine and guinea pig). In this assay, a fine tipped electrode impales a single myocyte within the exposed surface of the preparation, and records action potentials (AP). Changes in the morphology or duration of the AP will reflect pharmacological

effects of the drug studied on native cardiac channels. One added advantage of this approach is that the experimental conditions can be manipulated to simulate predisposing factors for QT prolongation and TdP. This assay is also useful because it allows drug effects on native cardiac channels to be studied, and can be used to detect effects mediated through pumps or exchange mechanisms, as well as ion channels other than the I_{Kr} channel. On the other hand, metabolites of a drug cannot be generated, and need to be tested in a separate series of experiences. Moreover, this assay is labor intensive, has a very low throughput, and failure to see APD prolongation in this model cannot exclude pro-arrhythmic toxicity or the risk of TdP in humans [28].

The other approach evaluates the effects of drugs on HERG current expressed in heterologous expression systems, or on native I_{Kr} current recorded from isolated cardiac myocytes, using the voltage clamp technique. This technique uses a single microelectrode to voltage-clamp the electrical potential difference across the cell membrane, while measuring the current carried by ions flowing through ion channels expressed in the cell membrane. No other method can provide such high quality and physiologically relevant data of precise and detailed activity of ion channel function. However, the predictive value of studies performed using this technique will depend on several factors including: selectivity of the compounds for HERG/I_{Kr} over other ion channels, concentration range studied, experimental conditions and protocols used. One of the major disadvantages of this assay is that it is technically difficult, and is low throughput. But this situation may be about to change, as high throughput planar patch-clamp technology is now emerging, and commercially available systems pledge to increase the rate of data acquisition dramatically to screen thousands of compounds per day [29].

A recent study investigated the utility of these two *in vitro* assays, and compared matched concentrations of a set of 10 structurally diverse drugs on APD changes in canine Purkinje fibers, and HERG channels stably expressed in human embryonic kidney cells [30]. The study highlighted the difficulty of assessing the potential pro-arrhythmic risk of drugs on the basis of concentration-dependent effects using these two assays. Moreover, it showed that, overall, the extent of HERG inhibition was poorly correlated with APD prolongation, consistent with the idea of additional drug effects on non-HERG channels. While HERG inhibition detected many of the drugs linked to QT prolongation and TdP, it was not fully predictive of pro-arrhythmic risk, as compounds, like verapamil, elicited significant HERG block with little effect on APD. Taken together, these results suggest that the HERG assay may occasionally oversimplify the drug effect on the repolarization process and that neither assay alone can adequately predict the pro-arrhythmic risk of drugs.

5.2. *In vivo* assays

In this assay, the ECG effects of a drug candidate are monitored in either conscious or anesthetized guinea pigs, rabbits, dogs or monkeys. Based on the survey of current practices, the dog seems to be the most popular species for *in vivo* studies. This seems to be justified mostly by the fact that the heart rate range is closer to humans than smaller animals, and by the similarities of the ionic makeup of Purkinje fibers and ventricular action potentials. The preferred methodology is the use of unrestrained animals

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.