Role of QT prolongation in drug discovery and development

Jitendra Narain Singh*, Piyush Agarwal and Shyam Sunder Sharma*

Department of Pharmacology and Toxicology,
National Institute of Pharmaceutical Education and Research (NIPER),
Sector 67, Phase 10, SAS Nagar, Punjab-160062, India
*dsssharma@niper.ac.in; jnsingh@niper.ac.in

Drug-induced inhibition of the cardiac rapidly delayed rectifier potassium channel I_{Kr} encoded by human ether-a-go-go-related gene (hERG) is considering the main concern in drug-induced arrhythmias. The in vitro screening method for an identification of the potential cardiac hERG liabilities is electrophysiology techniques; which are most reliable and gold standard technique. Recent developments in automated electrode-based voltage clamp promises sufficient throughput to support the needs of today's drug discovery programs. The new technology will allow significantly higher throughput and more scrupulous testing of new chemical entities (NCEs). Regulatory authorities from all over the world require data on the hERG assay of new drugs, as part of the cardiac safety evaluation process. The pre-clinical studies are generally conducted to evaluate the safety and efficacy of NCE, which will help in predicting the drug's likely risk/benefit assessment in new drug application process. The hERG assays became an integral component of regulatory requirements. In this review, we have summarized the brief highlights of the drug-induced QT prolongation and their withdrawal as well as regulatory aspects i.e. International Conference on Harmonization (ICH) S7B and ICH E14 guidelines in the process of drug discovery and development.

Introduction

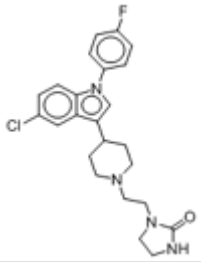
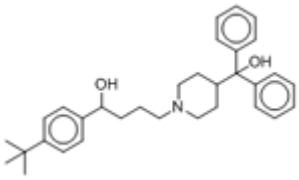
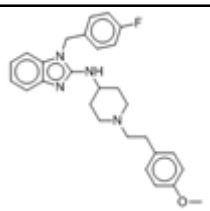
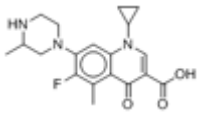
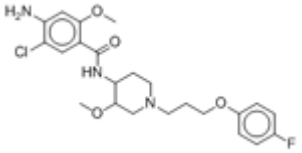
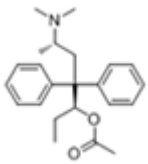
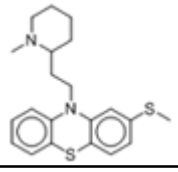
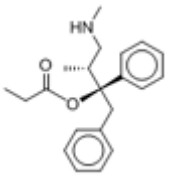
Delayed ventricular repolarization normally measured by prolongation of the electrocardiographic QT interval which is associated with an increased risk of ventricular arrhythmia, including *torsades de pointes* (*TdP*), resulting in fatal arrhythmias.[1,2] The delayed ventricular repolarization caused by a potential drug when given to patients for a long period is the major concern for the clinicians as well as pharmaceutical companies.[3-5] When QT interval is prolonged/ delayed by a potential drug and if it combined with other risk factors includes hypocalcaemia, hypokalaemia, myocardial ischaemia, diabetes, hypothyroidism, structural heart disease and congenital long QT interval syndrome and bradyarrhythmia may leads to ventricular tachyarrhythmia and torsades de pointes.[3-5]

The sudden death due to the human ether-a-go-go-related gene (hERG) channel blockade, as a side effect of non-antiarrhythmic drugs has been receiving increased regulatory attention.[6-8] Perhaps owing to the unique shape of the ligand-binding site and its hydrophobic character, the hERG channel has shown to interact with drugs with varying structure.[8] hERG channel is also classified as Kv11.1 channel, also has a toxic effects on the heart's rhythm.[8] Several *in silico* approaches have been attempted to predict hERG channel blockade. Some of these approaches aimed primarily at filtering out the potential hERG blockers in the context of virtual libraries; others involve understanding structure-activity relationships (SAR) governing hERG-drug interactions.

Drug Discovery Paradigm Shift

QT prolongation and sudden cardiac death has been observed with several drugs, due to this limitations during the last 20 years, large number of molecules have been withdrawn (Table 1). A manufacturer of a drug with a known or suspected QT prolongation potential will have an uphill battle to convince the regulators. Famotidine a highly selective histamine H_2 receptor antagonist[9,10] is indicated for the treatment of duodenal ulcer, gastric ulcer, gastroesophageal reflux disease, and Zollinger -Ellison syndrome.[9,10] It was estimated that about 18.8 million patients worldwide got satisfactory cure by famotidine.[9,10] It showed excellent tolerability profile during investigational trial and post marketing.[9,10] However, a patient with long QT syndrome caused by famotidine was reported in 1998.[11] The Ministry of Health, Labour and Welfare in Japan announced an official warning on the proarrhythmic potential of famotidine in September 2001, whereas there is still no evidence showing a causal link between famotidine administration, QT prolongation and onset of *TdP* in either human or animals. In 1966, Francois

Table.1. Drugs withdrawal from the market due to its cardiac arrhythmia effect

Drugs	Chemical Formula	Structure	Year of -(approval) withdrawal
Sertindole (Antidepressant)	$C_{24}H_{26}ClFN_4O$		(1998) 1998
Terfenadine (Antihistamine)	$C_{32}H_{41}NO_2$		(1985) 1998
Astemizole (Antihistamine)	$C_{28}H_{31}FN_4O$		(1988) 1999
Grepafloxacin (Antibiotic)	$C_{19}H_{22}FN_3O_3$		(1997) 1999
Cisapride (Prokinetic)	$C_{23}H_{29}ClFN_3O_4$		(1993) 2000
Levacetylmethadol (Analgesic)	$C_{23}H_{31}NO_2$		(1993) 2003
Thioridazine (Antipsychotic)	$C_{21}H_{26}N_2S_2$		(1988) 2005
Dextropropoxyphene (Analgesic)	$C_{21}H_{29}NO_2$		(1957) 2010

Dessertenne has described a specific electrocardiographic form of polymorphic ventricular tachycardia, which he termed as "torsades de pointes".[12] The word "torsades" refers to an ornamental motif imitating twisted hairs or threads as seen on classical architectural columns, and "pointes" referred to points or peaks.[12]

The inability of the food and drugs administration, (FDA) USA to effectively warn health care providers and patients about the drug interactions as well as our inability to translate the existing knowledge into changes in prescribing the right drug have resulted in huge economic consequences for the pharmaceutical industry and the loss from the market place of effective drugs, including terfenadine, mibefradil, astemizole, and cisapride. These four drugs were removed from the market or restricted in their use because it became clear that they

continue to be prescribed in an unsafe manner, even after multiple warning letters were disseminated by the manufacturer and the FDA to health care professionals concerning their proper use. Each of these drugs has value in the pharmaceutical marketplace, and each has value to patients. However, because the manufacturer and the FDA could not prevent co-prescription of these drugs with interacting drugs resulting in fatal interactions, the risk associated with continued widespread availability could not be justified.

Drugs withdrawn due to QT prolongation and *TdP*

Sertindole: It is a non-sedating a typical antipsychotic drug with its activity at dopamine and serotonin receptors in the brain. Chemically, it is classified as a phenylindole derivative and used for the treatment of schizophrenia.[13] Sertindole was developed by the Danish pharmaceutical company, Denmark and marketed under license by Abbott Labs, Abbott Park, Illinois, USA. In February, 1998, FDA withdrew this drug due to the increased risk of sudden death from prolongation of QT interval. In a trial of 2000 patients on taking this drug, 27 patients died unexpectedly, including 13 sudden deaths. Further, it was found to be associated with 19 ms QTc prolongation.[14]

Terfenadine: It was marketed by Sanofi-Aventis, France as an antihistamine drug, in 1985. It also blocks the $Kv_{11.1}$ channel. Due to its effect on $Kv_{11.1}$ channel it also has toxic effects on the heart's rhythm (e.g.ventricular tachycardia and *TdP*).[15,16] FDA removed it from the United States of America market in 1997. Terfenadine was a great breakthrough in the allergy arena, in that it treated the symptoms of allergic rhinitis without causing the drowsiness and fatigue like other antihistamines. Soon, it turned out that terfenadine, particularly when taken together with antibiotics or antifungal drugs could cause heart rhythm abnormalities.[16] Throughout its years on the market, the FDA has received about 40 reports of the abnormalities, linked to 8 deaths.[17] However, because it was the only antihistamine available without the side effect of drowsiness and fatigue, the FDA deemed that the benefits outweighed the risks of the drug. However, in 1996 the FDA approved another Hoechst Marion Roussel antihistamine, fexofenadine, which did not cause abnormal heart rhythms. That put terfenadine out of the category of benefits outweighing risks.[18] The FDA asked the company to withdraw the drug from the market because of its negative effects. Ultimately the FDA banned terfenadine in Jaunary 1997, leading to a recall.[19] Fexofenadine (the active metabolite of terfenadine), was approved in July 1996, after an unusually rapid development programme. Its introduction sets a new standard of safety that led the FDA to request the withdrawal of terfenadine because a safer version of an equivalent drug is now available.

Cisapride: It was discovered as prokinetic agent. It increases themotility in the upper GIT. The drug was withdrawn by FDA from the U.S. market on July 14, 2000 due to its arrhythmogenic potential.[20] In Sept.1993, to April 1996, the FDA's Med Watch reporting program received reports of 34 patients in whom *TdP* developed and 23 in whom prolonged QT intervals developed while using this drug. Four patients were reported to have died, and sixteen responded to resuscitation after cardio-pulmonary arrest.[21] Arrhythmia often preceded by the episodic syncope. Seven of the patients were children, and one was an adolescent. Cisapride shares Class III antiarrhythmic properties by blockade of distinct voltage-dependent potassium channels, thus prolongs the cardiac action potential duration, which delays the cardiac repolarization and prolong the QT interval. The incidence of adverse reactions which was found to be 1 per 12,000 to 1 per 120,000 patients causing drug-induced *TdP* in cohort pharmaco-epidemiological studies.[22] Cisapride can dose-dependently prolongs cardiac action potential duration of Purkinje fibres and ventricular muscle by blocking *hERG* potassium channel hence, reducing the rapid component of the cardiac delayed rectifier K^+ current (I_{Kr}).[23]

Astemizole: In 1977 astemizole, a second-generation antihistamine was discovered by Janssen Pharmaceutica, Belgium and marketed in 1988. It is metabolized to desmethylastemizole (DEA), similar to non-sedating second-generation anti-histamine (H_1-antagonist) drug which has been withdrawn from the market because of potentially fatal side effects (arrhythmias due to *hERG* channel blockade).[24] In June 1999, the FDA has determined that astemizole (10 mg tablets) should be withdrawn due to safety reasons. Although it has been removed from the market for inducing QT interval prolongation, now it has been reemerging as a potential anti-cancer and anti-malarial agent.[25] In patients receiving astemizole, doses exceeding or concomitant therapy with ketoconazole or macrolide antibiotics have been implicated in the increased risk of QT prolongation.

Grepafloxacin: Glaxo Wellcome, UK marketed Grepafloxacin in the name of Raxar in 1997. It is a fluoroquinolone anti-bacterial agent used to treat bacterial infections. It was withdrawn worldwide from markets in 1999, owing to its side effect causing prolongation of QT interval on the electrocardiogram, leads to sudden death. The rate for grepafloxacin

causing *TdP* is 3.8 per million and is associated with a mean QTc prolongation of 11 ms.[26]

Thioridazine: Thioridazine hydrochloride was approved in 1988 by Mutual Pharm, Philadelphia, USA. It was previously used in the treatment of schizophrenia and psychosis but now, it has been withdrawn worldwide in 2005 because of severe cardiac arrhythmias. Among the study of 154 person, about 29% of subjects were found to have 60 ms increase from the normal QTc interval.[27]

Levacetylmethadol: It was approved in 1993 by the FDA. It is a synthetic opioidas a second-line treatment for the treatment of opioid dependence, with a long duration of action. It has a similar structure like methadone. In 2001, it was removed from the European market due to reports of life-threatening ventricular rhythm disorders. Later in 2003, Roxane Laboratories, Inc. USA discontinued this drug in USA.[28]

Dextropropoxyphene: This drug was first approved by the FDA in 1957, propoxyphene is sold by prescription under various names both alone or in combination with acetaminophen. It is an analgesic in the opioid category. It was patented in 1955 and manufactured by Eli Lilly and Company, USA. It is an analgesic in the opioid category; which is used to treat mild pain, anti-tussive effect and local anaesthetic effects. It has been taken off the market from the USA on November 19, 2010 by the FDA due the fatal overdoses and heart arrhythmias.[29]

Mechanism of drug-induced QT prolongation

In the generation of the cardiac action potential in the cellular level, depolarization phase is driven by the sodium and repolarization phase is driven by potassium. Many types of the K^+ channels exist in the myocytes. In the repolarization of action potential the delayed rectifier K^+ currents current, I_{Kr} (rapid) and I_{Ks} (slow) are involved. Blockade of either of these delayed rectifier K^+ currents may prolong the action potential. I_{Kr} is the most susceptible to pharmacological influence.[30,31]

Drug-induced blockade of I_{Kr} current is responsible for the pro-arrhythmic effect. Blockade of the I_{Kr} current noticeable clinically as a prolonged QT interval and on the surface ECG by showing the abnormalities in T or U wave. The prolongation of repolarization of action potential may lead in subsequent activation of an inward depolarization current, known as an early after-depolarization (EAD), which may support triggered activity and after increased dispersion of repolarization, this may induce re-entry and provoke *TdP*, which is then sustained by further re-entry or spiral wave activity. Long QT Syndrome (LQTS) is a cardiac disorder in which otherwise healthy patients are susceptible to a cardiac arrhythmia (*TdP*) that manifests as fainting spells and sudden cardiac death. The molecular mechanism for drug-induced LQTS is now thought to be pharmacological blockade of the 'rapid' delayed rectifier potassium (K^+) current (I_{Kr}) in ventricular cardiomyocytes; at the molecular level this current is mediated by *hERG*, which is the α subunit of this channel.[32-34] However, there are a number of controversial issues as to how this works.[32-34]

Genesis of ICH S7B Guidelines

Need of Guideline ICH S7B for safety pharmacology studies were announced in 2002 based upon currently available information to assess the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals.[35] The health care professional, pharmaceutical manufacturer and regulator all shares a common interest in the development and use of drugs that optimize the risk/benefit ratio, in other words are effective, and have an acceptable level of undesirable effects. The detailed ICH S7B guidelines were published in October 2005 on appropriate levels and methods of pre-clinical testing (Fig. 1).[35,36]

In order to further understand the potential for a pharmacologically active drug to influence QT prolongation; initially predictions are made of a drug's likely pharmaceutical effects in man based on its chemistry and similarity to other known products. These predictions were further evaluated using *ex-vivo, in vivo, in vitro* and *in silico* models. After sufficient information from animal results has established and carefully evaluated the projections are made on the likely response in human. Once risk/benefit profile of candidate drugs is established. The safety study can be conducted on man by using relatively small single doses several logarithms lower than those used in animals, and they are carefully monitored across a range of physiological parameters than larger dose and duration treatments can be conducted in appropriate man studies. The dosing is gradually altered in amount and/or duration to assess for physiological responses (both beneficial and harmful), but any modification is always well within experience previously gained from animal models. Both the safety and efficacy of a candidate drug are assessed and depending on the nature of the drug and intended indication the scale of these studies may scale up to over six months exposure involving several thousand patients. To establish safety profile the regulator reviews the safety and efficacy data and may decide to approve the

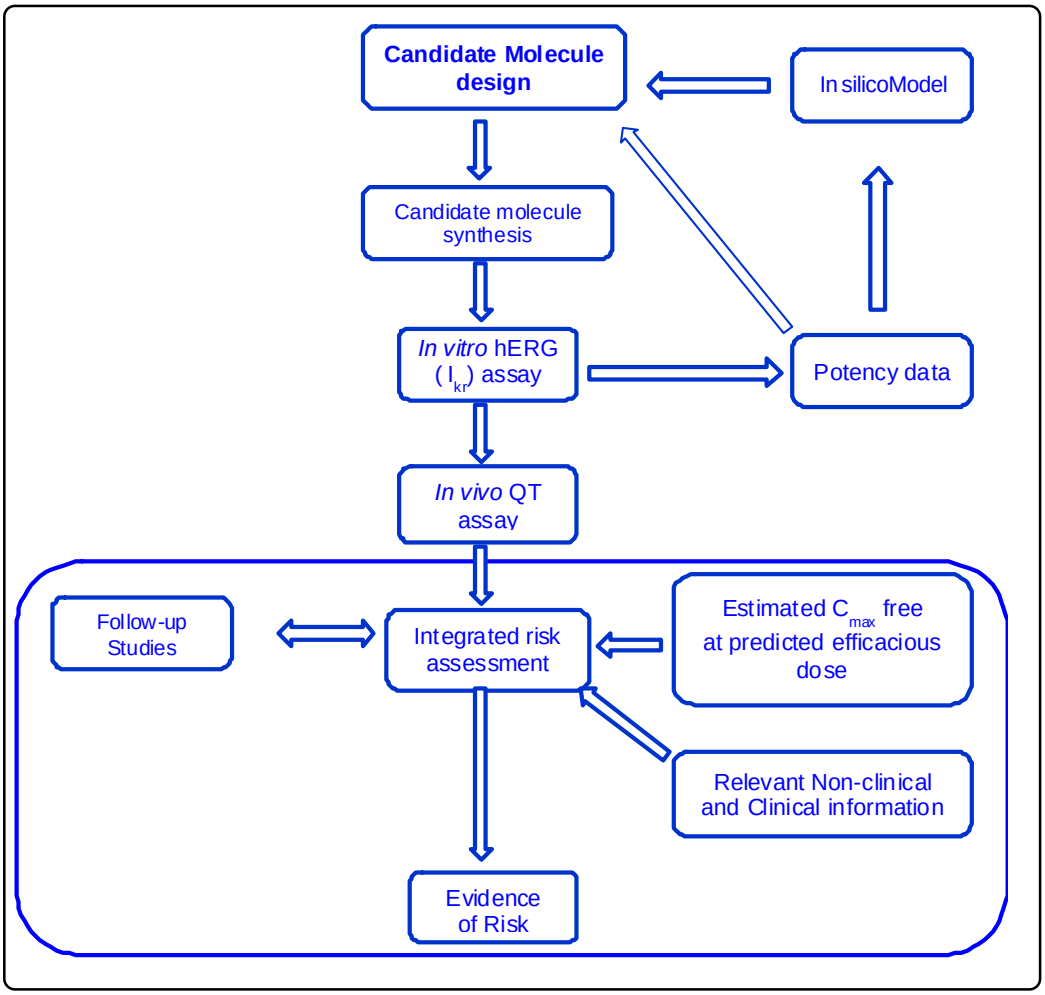


Fig.1. Schematic diagram of the drug discovery and development in relation to non-clinical QT strategy as per ICH S7B guideline.

product for marketed use. The pharmaceutical manufacturer is obliged to continuously monitor and react to the safety of its medicines as post marketing surveillance.

In performing these investigations many elements must be considered, for example the absorption, distribution, metabolism, and elimination characteristics of the candidate drug, single vs. repeat dosing, screening for active metabolites, the choice and number of appropriate animal models, serial vs. continuous electrophysiological monitoring, etc.[35,37-39] Two leading cardiologists have provided further guidance on the interpretation of abnormal results.[40] In these precautionary advices, it is suggested that none of the available pre-clinical models are superior to the other models in their capacity to predict an adverse clinical outcome. Further, if the candidate drug produces no abnormality in pre-clinical screening, it is also unlikely to produce a clinical QT prolongation effect. Even the candidate drug molecule produces abnormalities during pre-clinical screening may not, necessarily to produce a clinical effect. In these precautionary note it is also suggested that the false positives, as well as false negative results must be carefully considered and calculations show the inter- and intra-individual variations in phase I/II clinical trials.[40] According to Malik and Camm an ECG screening is quite specific and sensitive to determine the QT prolongation during early clinical studies.

These scientific advances have led to revisions to regulatory guidelines. For example, the FDA have issued a document "Answers to Frequently Asked Questions" (January 10, 2002) and are now recommending, effective for new trials from autumn 2002, that new drug sponsors submit ECG raw data that will allow the FDA to make its own judgements about ECG interval determinations and findings such as abnormal ECG morphology. They advise that such data needs to be provided in digital format with annotation.

Assessment of QT interval is a weak surrogate for *TdP*. QT prolongation is not the principle cause of *TdP* arrhythmias. Precise estimation of the extent of QT prolongation is now considered to be of poor scientific value, and it should not be expected to be of regulatory value for more than a few more years. Meantime, the relationship between heart rate and QT interval should be determined for each of the trial subjects. From the regulatory perspective, assessment of QT prolongation would continue to be used in the evaluation of *TdP* in the absence of a better clinical surrogate. Some regulators have expressed a willingness to accept new methodologies that are scientifically sound and adequately validated. For drugs already on the market, manufacturers need to continue to be vigilant.

6. ICH E14 guideline

There are some non-antiarrhythmic drugs which delay cardiac repolarization favoring the development of arrhythmias, which is marked by prolongation of the QT interval and induces potentially fatal ventricular tachyarrhythmias as a

result of which the vital milestone in the drug safety was reached when the regulatory guidelines are being adapted. The ICH issued E14 guidance -*The Clinical Evaluation Of QT/QTC Interval Prolongation And Proarrhythmic Potential For Nonantiarrhythmic Drugs,* was adapted on 12 May 2005 and was quickly implemented in Europe and in the United States.[41] The idea behind ICH E14 guidelines was an establishment of one single trial, the 'thorough QT/ QTc study' (typically conducted in healthy volunteers if a patient is found to be proarrhythmic), for the identification of the drugs that may cause QT prolongation.[42] Ideally, the major clinical studies require the study of female and elderly patients, as well as patients with co-morbidities and concomitant medications mainly the target population.[41,42] The study also evaluates the effect of the drug on all ECG parameters such as the T-wave morphology, heart rate and other cardiac intervals, so it is better termed as a 'thorough ECG trial' or TET, as first widespread by Morganroth.[43] The E14 recommendations are also applicable for the approved drugs when a new dose, route of administration or target population are explored. Moreover, the guideline is invalid for the products with highly localized distribution and those which are used topically and are not absorbed. Thus, lack of frequent correlation between the route of administration and systemic bioavailability.[41] In 2007, Internal Review Team (IRT) formed by the USFDA, which acts in an advisory function on QT assessment in other clinical trials. In the study design, crossover or parallel group study are being followed at high and sub-therapeutic dose through which concentration-response relationship for QT/QTc prolongation have been characterized to judge the pharmacokinetic and pharmacodynamic properties of the drug.[42]

To interpret the results, drugs that prolong the mean QT/QTc interval for more than the threshold value 5 ms are likely to cause *TdP*. Further, such patients should be evaluated for the additional risk factors (like hypokalemia, etc.) that may lead to these events. Before interpretation, the QT interval is analysed, which bears an inverse relationship to heart rate and the measured QT intervals are corrected for heart rate to judge the prolongation of QT interval relative to baseline for which the suggested correction formulae are mainly Bazett's and Fridericia's formula. The timing of the TQT study must be before crucial clinical trials, for these study moxifloxacin (400 mg), a fluoroquinolone antibiotic is employed as a positive control which prolongs QT interval in a range of 8 to 15 ms with well established assay sensitivity thus detects even a small effect on the QT interval.[43] The total number of TQT studies, exceeds 100 at 'FDA ECG warehouse' to which all electrocardiograms (ECGs) must be submitted as annotated *xml* files, including few of the results have been shared publicly.[44]

Test systems

In silico modeling

The *in silico* approach is also included in the guidelines to eliminate the potential NCE's responsible for the hERG blockade during the early drug discovery process. There are three related models : classification method, homology modeling and QSAR models.[45]

Homology model- In this model, 3D structure of protein is created, the understanding of the pore structure of *hERG* channel is known through the crystal structure of the bacterial K^+ channel KcsA (closed) and MthK (open).[46,47]

QSAR model- The model represents a computational approach, to relate activity data of molecules to a set of molecular descriptors thus explains the absolute activity of compounds.[45,48] Classification model- This represents an approach to distribute the molecules into categories of the same type (active and inactive)[45] by Generative Topographic Maps (GTM, derives probability density functions and prior probabilities) and Support Vector Machines (SVM, estimate posterior probabilities) classification models.[49]

In vitro assays

Most pharmaceuticals are associated with *TdP* owing to the inhibition of the rapidly activating delayed rectifier current, I_{Kr}. Therefore, particular attention assays for I_{Kr} is prudent for assessing the risk of QT interval prolongation. Inhibition of other outward (repolarizing) ionic currents (e.g., I_{to}, I_{K1}, and I_{Ks}) or increases in inward (depolarizing) ionic currents (e.g., I_{Na}) could also lead to QT interval prolongation and, therefore, should be considered when investigating the mechanism(s) for QT interval. Using the voltage clamp technique, outward or inward ionic currents can be measured from various single cell preparations. Because of inherent difficulties associated with the I_{Kr} recordings in native myocytes, much of the available data for this current can be obtained using recombinant cell lines expressing *hERG*. Hazard identification and mechanism of action studies (e.g., voltage-dependence, state-dependence of block and channel kinetics) are conveniently performed in heterologous expression systems because there are fewer types of endogenous currents present. The relative potency of ionic current inhibition is most often expressed as the 50% inhibitory concentration

(IC_{50}). The slow decrease in current that is often observed during whole cell voltage clamp ("rundown") can make it difficult to accurately measure IC_{50} values. It should also be noted that some pharmaceuticals show time, voltage or channel state dependent interactions with channels that can influence the determination of IC_{50} values.

In vivo assay

Conscious anesthetized non-rodents (dog, monkey, swine, rabbit, ferret, and guinea pig) are increasingly being studied for *in vivo* electrophysiology studies[31]. The QT interval of the ECG is used as the endpoint to test the effects of a test substance on ventricular repolarization. The information regarding the ventricular repolarization (e.g., monophasic action potential duration and effective refractory period) is obtained from *in vivo* models. Additional safety parameters like blood pressure and heart rate is also evaluated simultaneously. Ideally, QT interval data obtained after administration of a test substance must be compared with control and baseline data at similar heart rates. Usually, corrected QT interval for heart rate (QTc) is calculated using formulae such as Bazett or Fridericia.[35] Also, potential neuronal and hormonal influences on the pharmacodynamic effect of the pharmaceuticals are present in animals.[50]

Concluding remarks

Drug-induced QT prolongation is one of the major setbacks for pharmaceutical industry. About eight drugs have already been withdrawn from the market. ICH guideline S7B and E14 had already laid down.[36,41] The goal of clinical electrophysiology trials must be to determine the maximal possible QT prolongation attributable of the drug can be study, and to define the circumstances under which such prolongation can be achieve. Pre-clinical electrophysiological findings will not often be the proper basis of go/no-go decisions.

References

1. Hondeghem LM, Cardiovasc Res. (2000), 45, 57-60.
2. Sanguinetti MC, Jiang C, Curran ME, et al., Cell. (1995), 81, 299-307.
3. De Ponti F, Poluzzi E and Montanaro N, Eur J Clin Pharmacol. (2001), 57, 185-209.
4. De Ponti F, Poluzzi E, Montanaro N, et al., Lancet. (2000), 356, 75-76.
5. Tamargo J, Jpn J Pharmacol. (2000), 83, 1-19.
6. Bezanilla F, Nat Rev Mol Cell Biol. (2008), 9, 323-332.
7. Swartz KJ, Nat Rev Neurosci. (2004), 5, 905-916.
8. Warmke JW and Ganetzky B, Proc Natl Acad Sci. U S A. (1994), 91, 3438-3442.
9. Howden CW and Tytgat GN, Clin Ther. (1996), 18, 36-54; discussion 35.
10. Langtry HD, Grant SM and Goa KL, Drugs. (1989), 38, 551-590.
11. Endo T, Katoh T, Kiuchi K, et al., Am J Med. (2000), 108, 438-439.
12. Yap YG and Camm AJ, Heart. (2003), 89, 1363-1372.
13. Leucht S, Cipriani A, Spineli L, et al., Lancet (London, England). (2013), 382, 951-962.
14. Nielsen J, Wang F, Graff C, et al., Therapeutic Advances in Psychopharmacol. (2015), 5, 26-31.
15. Monahan BP, Ferguson CL, Killeavy ES, et al., JAMA. (1990), 264, 2788-2790.
16. Rajput SK, Singh JN and Sharma SS, Pharmacol Rep. (2010), 62, 683-688.
17. Figueiras A, Tato F, Fontainas J, et al., Medical Care. (1999), 37, 809-814.
18. Eland IA, Belton KJ, van Grootheest AC, et al., British J Clin Pharmacol. (1999), 48, 623-627.
19. Chyka PA and McCommon SW, Drug Safety. (2000), 23, 87-93.
20. Mohammad S, Zhou Z, Gong Q, et al., The American J Physiol. (1997), 273, H2534-2538.
21. Drolet B, Khalifa M, Daleau P, et al., Circulation. (1998), 97, 204-210.
22. Layton D, Key C and Shakir SA, Pharmacoepidemiology and Drug Safety. (2003), 12, 31-40.
23. Kii Y and Ito T, J Cardio Pharmacol. (1997), 29, 670-675.
24. Bishop R and Gaudry P, Archives of Emergency Medicine. (1989), 6, 63-65.
25. Garcia-Quiroz J and Camacho J, Anti-cancer Agents in Medicinal Chem. (2011), 11, 307-314.
26. Rubinstein E and Camm J, The J Antimicrobial Chemotherapy. (2002), 49, 593-596.
27. Huffman JC and Stern TA, Primary Care Companion to the J of Clinical Psychiatry. (2003), 5, 278.
28. Stringer J, Welsh C and Tommasello A, American J Health-System Pharmacy (AJHP : official journal of the American Society of Health-System Pharmacists). (2009), 66, 825-833.
29. Raffa RB, Burmeister JJ, Yuvasheva E, et al., Expert Opinion Pharmacotherapy. (2012), 13, 1397-1409.
30. Singh JN, Kumar S, Berhe AH, et al., Curr Res Infor Pharm Sci. (2007), 8, 6-12.
31. Singh JN and Sharma SS (2011). hERG Potassium Channels in Drug Discovery and Development. Springer. pp 149-190.
32. Witchel HJ and Hancox JC, Clin Exp Pharmacol Physiol. (2000), 27, 753-766.
33. Witchel HJ, Hancox JC and Nutt DJ, J Clin Psychopharmacol. (2003), 23, 58-77.
34. Witchel HJ, Hancox JC, Nutt DJ, et al., Br J Psychiatry. (2003), 182, 171-172.
35. Bode G and Olejniczak K, Fundamental & Clinical Pharmacology. (2002), 16, 105-118.
36. Guideline ICHHT, S7B (*http://www. ich.org/products/ guidelines/safety/article/ safety-guidelines. html*). (2005),
37. Abi-Gerges N, Philp K, Pollard C, et al., Fundamental & Clinical Pharmacol. (2004), 18, 139-151.
38. Cavero I and Crumb W, Expert Opinion on Drug Safety. (2005), 4, 509-530.
39. Friedrichs GS, Patmore L and Bass A, J Pharmacol Toxicol Methods. (2005), 52, 6-11.
40. Malik M and Camm AJ, J Am Coll Cardiol. (1993), 22, 566-568.
41. Shah RR, Drug Safety (2005), 28, 1009-1028.
42. Darpo B, Bri. J Pharmacol. (2010), 159, 49-57.
43. Demolis JL, Kubitza D, Tenneze L, et al., Clin Pharmacol Therap. (2000), 68, 658-666.
44. Sarapa N, Expert Opinion on Drug Safety. (2007), 6, 595-607.
45. Aronov AM, Drug Discovery Today. (2005), 10, 149-155.
46. Doyle DA, Morais Cabral J, Pfuetzner RA, et al., Science (New York, N.Y.). (1998), 280, 69-77.
47. Jiang Y, Lee A, Chen J, et al., Nature. (2002), 417, 515-522.
48. Keseru GM, Bioorganic & Med. Chem. Lett. (2003), 13, 2773-2775.
49. Kireeva N, Kuznetsov SL, Bykov AA, et al., SAR and QSAR in Environmental Res. (2013), 24, 103-117.
50. Pollard CE, AbiGerges N, Bridgland-Taylor MH, et al., Brit J Pharmacol. (2010), 159, 12-21.