

Cardiac Drug Safety and hERG channel

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Cardiac safety and QT prolongation

In recent years, multiple blockbuster drugs such as terfenadine (Seldane), cisapride (Propulsid) have been pulled out of the market due to a lethal side effect of drug on human heart. These drugs had caused cardiac arrhythmia called torsade de pointes. In the past decade, the single most common cause of the drug withdrawal (or additional restriction of the usage) is the side effect of prolongation of the QT interval associated with polymorphic ventricular arrhythmia or torsade de pointes. In almost all the cases, the potentially lethal side effect was induced by the drug interacting with a cardiac potassium channel, named hERG.

In order to protect clinical trial participants and patients, International Conference of Harmonization (ICH) published a guideline (S7B) in 2002 to suggest all new drugs being tested preclinically for cardiac safety and hERG sensitivity before submitting for regulatory reviews. This review will discuss long-QT syndrome, drug effect on the cardiac function and cardiac ion channels as well as assays for screening leading compounds for cardiac toxicity.

What is long-QT syndrome (LQTS)

In electrocardiograms (ECGs), the QT interval refers to the time from the beginning of the QRS complex to the end of the T wave. In order to correct for different heart rates, long QT (LQT) abnormality is defined as a QTc (QT interval corrected for the heart rates) greater than 460 ms (figure 1).

$$QTc = QT / \sqrt{RR}$$

where RR is the time between two heart beats (R-R interval), measured in seconds.

QT interval prolongation can be congenital (long QT syndrome) or acquired (drug-induced long QT). Long QT syndrome is a cardiac disorder that causes synope, seizures and even sudden death. The root causes of these clinical manifests are ventricular tachyarrhythmias, specifically torsade de pointes, which frequently degenerate into ventricular fibrillation. There are a number of genetic mutations that induce LQT. To date, the congenital LQT has been recognized in two forms: autosomal dominant LQT mutations cause Romano-Ward syndrome and autosomal-recessive LQT mutations cause Jervell and Lange-Nielsen syndrome¹². At least five LQT genes have been identified for autosomal-dominant LQT. Two genes so far have been identified to cause autosomal-recessive disorder. Table 1 summarizes these genes and their coded proteins.

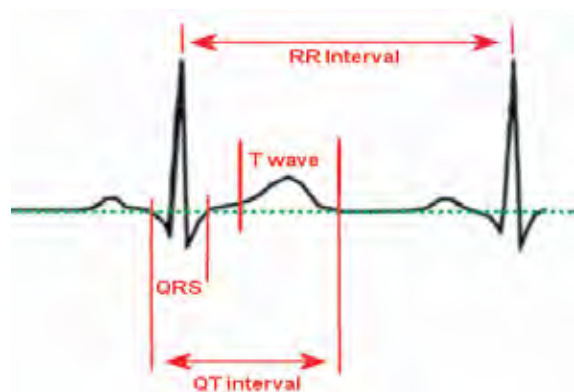


Figure 1 Schematic ECG waveforms and QTc calculation. QT interval and RR interval are labeled red.

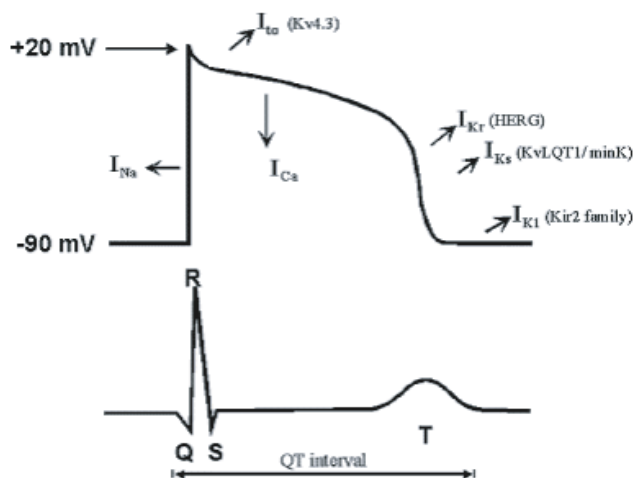


Figure 2 Action potential (AP) of a cardiac myocyte (top) and whole heart ECG (bottom). The depolarization of myocytes correlates to the starting of QRS complex of ECG. And the repolarization of myocytes forms the T wave on ECG. Corresponding ionic currents underlining myocyte action potential was noted.

Table 1 Genes causing congenital LQTS

LQT classification	Gene	Chromosome location	Ion channel protein
LQT1	KCNQ1	11p15.5	KVLQT1 (Kv7.1) Iks
LQT2	KCNH2	7q35-q36	hERG (Kv11.1) Ikr
LQT3	SCN5A	3p24-p21	Na(v) 1.5
LQT4	ANK2	4q25-q27	Ankyrin-B, accessory protein
LQT5	KCNE1	21q22	Min K, Iks subunit
LQT6	KCNE2	21q22	MiRP1
LQT7	KCNJ2	17q23	Kir2.1
LQT8	CACNA1C	12p13.3	Ca(v) 1.2

Mutations in the KCNE1, KCNE2, KCNH2, KCNQ1, and SCN5A ion channel genes cause Romano-Ward syndrome. The ANK2 non-ion channel gene is also associated with Romano-Ward syndrome. Mutations in the KCNE1 and KCNQ1 genes cause Jervell and Lange-Nielsen syndrome.

KVLQT1, Min K, HERG, MiRP1 and Kir2.1 are potassium channels or its subunits expressed in the heart. SCN5A encodes cardiac sodium channel Na(v)1.5. CACNA1C encodes L type calcium channel Ca(v)1.2. And ANK2 encodes a protein called neuronal type ankyrin, which helps ion channel protein's insertion into the membrane. So most, if not all of the genetic mutations, alter the function of cardiac ion channels. This is not surprising because the LQT disorder is manifested first as an ECG abnormality. And the underlying molecular mechanisms of ECG are cardiac ion channel activities (see figure 2 clinical whole heart ECG and single myocyte ECG).

Although there have been just eight LQT genes discovered, a large number of mutations of these genes have been found in patient populations. A registry of LQTS mutations can be found at the following website: <http://www.fsm.it/cardmoc/> New entries are constantly being added to the list.

Torsade de pointes

With a prolonged QT interval, there is an increased risk of ventricular tachyarrhythmia, including a specific form, called Torsade de pointes (TdP). Torsade de pointes (from the French, means “twisting of the points”) is a polymorphic ventricular tachycardia seen at the onset of QT prolongation. The ECG pattern of Torsade de pointes is distinctive, sometimes being described as a sine wave within a sine wave. It is called “twisting” because when the peaks are at their smallest in one ECG lead, they are at their largest in another, as if the several ECG leads were different views of a vibrating string whose plane of vibration was being slowly rotated around the axis of the string.

Triggers in LQTS

LQTS happens rarely, and usually being a consequence of some triggering events. But it is a serious threat to patients’ health and life. Some of its triggering factors are listed below:
 Sports: such as swimming, running
 Startle: an alarm clock, a loud horn, a ringing phone

Emotions: anger, crying, test taking or other stressful situations

Other: sudden death may also occur during sleep

Medication: some medications can also trigger LQTS.

Drug-induced LQTS

Besides the genetically coded, inherent LQT abnormalities, there are also so called “acquired LQTS”, which refers to drug-induced LQTS in patients with healthy genetic background. This drug-induced LQTS can lead to serious drug side effect, sometime even as severe as patient sudden death. Thus, drug-induced LQT has become a serious concern for pharmaceutical research.

Some marketed antiarrhythmic drugs, such as dofetilide (Tikosyn), quinidine (Cardioquin), and sotalol (Betapace), induce TdP in a small number of patients who receive them. This is not surprising since those drugs were meant to interfere with the cardiac electrophysiology. Those drugs, in general, are more beneficial to patients than the potential side effect caused by them.

However, there are drugs, which were designed for targets having no connection with cardiac function at all that induce TdP. The total incidence of drug induced TdP is largely unknown. But the absolute total number of reports is relatively low. Between 1983 and 1999, 761 cases of TdP, of which 34 were fatal cases, were reported to the World Health Organization Drug Monitoring Center by member states¹³. Although the occurrence of such side effect is rare, a few lethal cases are serious enough to cause withdrawal of those drugs from the market. Since 1999, a number of these drugs have been withdrawn, including terfenadine (Seldane, antihistamine for allergies) astemizole (Hismanal, antihistamine for allergies), cisapride (Propusid for GI stimulation) etc. Some other drugs such as ziprasidone (Geodon for antipsychotics), though not withdrawn from the market, have undergone severe labeling restrictions¹.

The number of drugs inducing LQTS or TdP has been growing. Close monitoring of these drugs are needed for patient medication. A list of these drugs labeled with various risk levels can be found at the following website:

<http://www.qtdrugs.org/medical-pros/drug-lists/drug-lists.htm>

Since ECG is composed by a family of ionic currents, in principle, drugs can induce LQTS through a variety of means. In practice, however, virtually every case of drug-induced LQTS and torsades de pointes can be traced back to one specific mechanism: blockade of a specific cardiac potassium channel called hERG. Therefore, the interaction between hERG chan-

nel and drug candidate represents an important safety concern for the pharmaceutical industry. Because of this, screening of drug candidate on hERG is quickly becoming an industrial standard practice.

hERG channel, a molecular brake

What is hERG?

The human Ether-a-go-go Related Gene (hERG) encodes a voltage-gated potassium channel expressing in cardiac myocyte. It functions as an inward rectifier channel (preferably allow potassium ions to flow into the cell under balanced ionic concentration)¹¹. But under physiological ionic concentrations, most of the potassium currents are flow outward. The channel name derived from a drosophila potassium channel gene, called ether-a-go-go. A mutation of this fly gene causes drosophila to dance like a go-go dancer when being exposed to ether.

hERG gene encodes a cardiac potassium channel that functions as a molecular brake for cardiac action potential. hERG expresses predominantly in the heart. It plays a critical role of regulating the heart beat. Physiologically, its expression underlines the rapid delayed rectifier current, IKr (figure 2), while IKr is the major driving force of the repolarization of cardiac myocytes.

The hERG ion channel belongs to the voltage-gated ion channel superfamily of membrane proteins. There are around 400 pore forming ion channel genes in human genome. Based on current estimations for the total number of genes in human genome, ion channels correspond to about 1.3% of the total genes. Ion channels are the third largest class of genes for drug discovery research, trailing in number only kinases and G-protein-coupled receptors (GPCRs). hERG channel, not being considered as a drug development target, is becoming an important toxicology target.

Structure and biophysical properties of hERG

hERG channel belongs to the voltage-gated potassium channel family of genes. The gene translates into a protein consists of six transmembrane domains, called a subunit. The fourth transmembrane segment forms the voltage sensing machinery. The loop between segment five and six lines the interior of the channel. Like other voltage-gated potassium channels, hERG channels are tetramers. Four subunits form a functional ion channel pore. Figure 3A shows a schematic transmembrane topology of a single hERG subunit.

One unique property of hERG channel under physiological conditions is that it conducts large inward current upon

membrane repolarization. Like most of the voltage-gated ion channels, hERG channels activate (open) upon depolarization. But the channel quickly goes to the inactivated state under continued depolarization (figure 3B). So at the plateau phase of a cardiac action potential, there will not be significant amount of current following through the channel. When myocytes repolarize, the channel protein dwells in the open state before closing, hence a large outward current at the end of cardiac action potential, which helps to repolarize the cell quickly (figure 2 top). Any blockade of hERG currents will cause a prolonged plateau phase of action potential and cause QT prolongation. Figure 3C shows a typical hERG current recorded under voltage-clamp. The large outward tail current elucidated by membrane repolarization is characteristic of hERG channel.

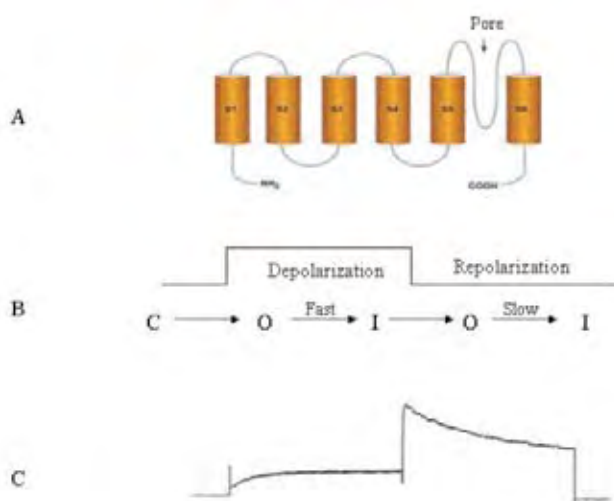


Figure 3 A. Structure of hERG channel subunit (model). B. The biophysical property hERG and C. A typical current profile under patch-clamp recording. Although hERG is classified as an inward rectifier, it does conduct outward current too, as shown in this figure.

hERG channel is infamous for its promiscuousness of drug interaction. There are a large collection of drugs and compounds known to interact and block channel activity. The majority of aforementioned LQT-inducing drugs are in fact hERG blockers. A three dimensional structure model of hERG reveals a hydrophobic pocket near the mouth of the internal opening of the channel. This pocket is very unique compared to other similarly structured potassium channels. It is believed that this pocket plays a critical role in drug-channel interaction.²

Assaying compound safety on LQT

To avoid the fatal side effect of drug induced LQT, International Conference of Harmonisation (ICH) has set up two guide lines for drug development in different stages. Section S7B was set for cardiac safety assessment during preclinical drug development. Section E14 was set for cardiac safety assessment during early clinical trials. Although the guide lines are not mandatory, all major pharmaceutical and biotech companies have adopted them and started testing drug-induced LQT and/or drug blockade of hERG channels prior to the starting of human clinical trials. As a matter of fact, due to the high costs of failing a lead compound in the late stage of drug discovery and development, more and more companies are moving the hERG assay to early stages of drug development.

In vivo assays for LQT

Drug-induced LQT can be assayed in vivo, employing either the whole animal or isolated perfused heart. Alternatively, drug-induced LQT can also be estimated by testing the drug in vitro, assaying the drug on recombinant hERG channels. In vivo studies can employ either whole animal or whole heart. Intact animal models allow investigation of ventricular repolarization or associated arrhythmias where integrated effects on the full complement of ion channels and all other physiological inputs are assessed. Whole animal studies are usually performed on dog or rabbit¹⁴. Sometimes Zebra fish can also be utilized to assess drug's cardiac effect⁸.

In vitro assays for LQT

Isolated perfused heart can be used directly to assay the QT prolongation. Since ECG and QT intervals can be measured from isolated spontaneously beating hearts, data so generated can be used to access the drug effect on heart-rate corrected QTc. Comparing with whole animal approach, an isolated heart eliminates physiological input from other parts of the system. So it's a simpler and more controlled approach. Recently, there are reports from pharmaceutical researchers using isolated guinea pig hearts to screen for potential cardiac side effect of drug candidates³.

In vitro assay for action potential duration (APD)

Multi-cellular tissues such as papillary muscles and Purkinje fibers have also been used to evaluate the drug effect. Action potentials (AP) from these tissues can be recorded and drug effect on AP can be directly monitored. APD assay with these cultured tissue provide a good mixture of physiological closeness to the in vivo environments and easy of control of the experiment.

To further simplify the model, APD from dissociated individual cardio myocyte can also be monitored to evaluate the drug effect. Compared with the recombinant hERG channel assay as discussed below, dissociated tissues and myocytes have the advantage of having all relevant physiological currents. Thus they are suitable for assessing effects on both action potential duration and physiological ionic currents.

Assaying compound activity on hERG

Since hERG is the single most common contributor of LQT and TdP, a compound's potential of inducing TdP and LQT can be directly evaluated with hERG channel in vitro and in recombinant expression systems. There are many traditional methods for assaying ion channels, as well as some newly developed technologies. Most of traditional ion channel assay methods can be applied to hERG assays. The most commonly used high throughput assays in the pharmaceutical industry are discussed below.

Radioligand-displacement assay

Since most of the hERG ligands occupy the same binding site on the channel protein, a ligand displacement assay can be designed. A [H3]-labeled ligand, such as dofetilide⁶ or astermizole is used to pre-incubate with cells or membrane fractions containing the ion channel protein complex. The compounds being assayed can then be applied to the cells or membrane fractions. Assay compounds will compete for the binding site with the radio-labeled ligand ([H3]-dofetilide). The competitive binding obeys mass-action law. By knowing the binding affinity of the labeled ligand, the binding affinities of assay ligands can then be calculated²⁴.

Flux assay

One way to measure the ion channel activity is to trace the amount of ions flow through the channel. Since rubidium ions can permeate through most potassium channels, hERG channel activity can be assayed using rubidium as a tracer, as well. Radioactive ⁸⁶Rb⁺ was used in the past. But the strong radioactivity made it less favorable in the industry. Instead, an atomic absorption spectrum measurement of non-radioactive ⁸⁷Rb⁺ assay was developed by Bayer AG¹⁰ and later commercialized by Aurora Biomed (Canada) with the ICR 8000/12000 systems.

The flux assay measures the end results of ions flowing through the open channel. It is a fast and economic way of measuring ion channel activity. The limitation of the assay is that no kinetic information of channel activity can be derived.

Membrane potential assay

Ion channel activity induces cell membrane potential changes. Monitoring the membrane potential of a cell can deduce additional kinetic information of channel activity. In the case of hERG, activation of the channels will repolarize the cell membrane, just as any potassium channel does. Fluorescent membrane potential indicators, e.g. Oxonols, DiBACs, or other dyes can be employed on FLIPR® (Molecular Devices, USA) or VIPR® (Aurora Discovery, USA) systems to indicate channel activity. In general, membrane potential dye based assays have the advantage of high throughput and low cost per data point. But it also comes with high rates of false positives and false negatives.

Electrophysiology and voltage-clamp assay

Ion channel activity can also be directly assayed by recording ionic current flow through ion channels by electrically controlling the cell membrane potential (voltage-clamp the cell membrane). Voltage-clamp technology, a very laborious process requiring a skilled operator and specialized instruments, was invented in the 1950's. It gained wide acceptance in the 1980's. Until now, it is still considered the "gold standard" of ion channel assays.

Drug efficacy on hERG channels can be assayed on ion channel bearing mammalian cell lines (common parental cell lines such as CHO-K1 or HEK-293), or *Xenopus* oocytes transiently expressing the ion channel gene through mRNA injection. Limitations of traditional electrophysiology is inherent to its complicated assay set ups and assay procedures. It's slow, labor-intensive, and hence expensive too.

Automatic (high throughput) electrophysiology

To address the limitations of manual voltage clamping technology, a number of automatic electrophysiology technologies have been introduced in recent years. Most of these technologies are based on planar electrodes, which include marketed products such as IonWorks® Quattro™ and PatchXpress® 7000A systems from Molecular Devices Corporation (USA), QPatch™ 16/48 system from Sophion AG (Germany), Cytospatch™ 100 from Cytoceptrix (Germany) and NPC®-16 from Nanion Technologies (Goffering a unique solution for automatic patch clamping is Flyion. It marketed an inverted patch clamp system employs a pipette tip, back filled with suspension cells to achieve automatic seal formation. A comparison of the throughputs and costs of the available automatic electrophysiology systems is listed in table 2⁵. In general, these automatic patching systems provide assay values show good correlation with that collected with traditional manual patch clamp systems⁷.

Table 2 Comparison of planar Patch Clamp systems

Vendor	Product name	# of assay /chip	Chip cost (US\$)	Datapoints /8HR day	Cost (US\$) / datapoint
Cytocentrics	CytoPatch™ 100	1	10	134	14
Flyion	Flyscreen® 8500	1	5	100~500	7.5~10
Molecular Devices	IonWorks® Quattro™	384	240	2300	0.75
Molecular Devices	PatchXpress® 7000A	16	130	240	8.13
Nanion	NPC® -Port-a-Patch	1	5	40	5

Besides these mammalian cell patch clamping systems, there are also a few automatic *Xenopus* oocyte based two-electrodes voltage-clamp systems. Molecular Devices is marketing an eight channel OpusXpress® 6000A system and Multi Channel Systems (Germany) is currently marketing a desktop solution, called Roboocyte®, which also has built-in mRNA injection function. Oocyte systems have their own unique applications, such as working with transiently expressing RNA- injected oocytes. This eliminates the relatively long process of generating stable cell lines. Besides this advantage, the oocyte system is also known to be easier to assay mutated ion channels.

Conclusion

Cardiac safety pharmacology and hERG liability is quickly becoming a must-have data point in the toxicity studies. More and more pharmaceutical and biotech companies are moving the hERG liability assay to the early stage of the discovery pipeline. As the assays become faster, cheaper and more reliable, it makes financial sense to screen any potential lead compounds before even getting into in vivo animal testing.

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