Cardiac hERG K^+ Channel as Safety and Pharmacological Target

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Abstract

The human *ether-á-go-go related* gene (*hERG*, *KCNH2*) encodes the pore-forming subunit of the potassium channel responsible for a fast component of the cardiac delayed rectifier potassium current (I_{Kr}). Outward I_{Kr} is an important determinant of cardiac action potential (AP) repolarization and effectively

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controls the duration of the QT interval in humans. Dysfunction of hERG channel can cause severe ventricular arrhythmias and thus modulators of the channel, including hERG inhibitors and activators, continue to attract intense pharmacological interest. Certain inhibitors of hERG channel prolong the action potential duration (APD) and effective refractory period (ERP) to suppress premature ventricular contraction and are used as class III antiarrhythmic agents. However, a reduction of the hERG/I_{Kr} current has been recognized as a predominant mechanism responsible for the drug-induced delayed repolarization known as acquired long QT syndromes (LQTS), which is linked to an increased risk for "torsades de pointes" (TdP) ventricular arrhythmias and sudden cardiac death. Many drugs of different classes and structures have been identified to carry TdP risk. Hence, assessing hERG/I_{Kr} blockade of new drug candidates is mandatory in the drug development process according to the regulatory agencies. In contrast, several hERG channel activators have been shown to enhance I_{Kr} and shorten the APD and thus might have potential antiarrhythmic effects against pathological LQTS. However, these activators may also be proarrhythmic due to excessive shortening of APD and the ERP.

Keywords

Activator · Arrhythmia · hERG · Inhibitor · Long QT syndromes

1 Introduction

Cardiac arrhythmias are one of the major causes of cardiovascular disease-related deaths worldwide. Ion channels are pore-forming proteins that provide pathways for the transmembrane movement of ions and thus control the cardiac action potential (AP) generation and propagation, resulting in the release of Ca^{2+} from intracellular stores and triggering cardiac muscle contraction. Abnormalities in cardiac ion channel function may lead to arrhythmias and sudden cardiac death (Keating and Sanguinetti 2001). The human *ether-á-go-go related* gene (hERG, *KCNH2*) encodes the pore-forming subunit (Kv11.1) of the channel that in cardiac myocytes conducts the rapidly activating delayed rectifier potassium current (I_{Kr}). Outward I_{Kr} is a critical current in the phase 3 AP repolarization in the human ventricle and effectively controls the QT interval of the electrocardiogram (Sanguinetti et al. 1995). Inhibition of I_{Kr} results in the prolongation of repolarization, which has been described as an antiarrhythmic mechanism of Class III antiarrhythmic agents (Singh and Vaughan Williams 1970). However, these drugs have also been found to be associated with an increased risk of arrhythmias. In addition to antiarrhythmic agents, a wide variety of different classes of non-antiarrhythmic pharmaceuticals have the potential to inhibit hERG/I_{Kr} current and, thus, can pose a threat of the drug-induced form of acquired long QT syndromes (LQTS) associated with an increased risk of an unusual life-threatening form of arrhythmia known as torsades de pointes (TdP) (Sanguinetti and Tristani-Firouzi 2006; Vandenberg et al. 2012). Consequently, assessing potential I_{Kr}/hERG inhibition of drug candidates has become a

major requirement in new drug development process (Hancox et al. 2008; Sanguinetti and Mitcheson 2005). Considerable effort has been made to understand the molecular basis underlying the susceptibility of hERG channel to pharmacological inhibition. A recent cryoelectron microscopy (cryo-EM) structure of hERG (Wang and MacKinnon 2017) has provided opportunities to better understand hERG channel gating and pharmacology (Butler et al. 2019). This review briefly describes hERG channel as a pharmacological and safety target for antiarrhythmic/proarrhythmic actions of drugs.

2 Structure of hERG Channel

Like other Kv channels, hERG channel is formed by co-assembly of four α subunits. Each α subunit has six transmembrane spanning α-helical segments (S1–S6) along with the intracellularly located N- and C-terminus. The voltage sensor domain (VSD) that senses transmembrane potential is formed by S1–S4 helices (Piper et al. 2003; Subbiah et al. 2004). S4 helix contains positively charged amino acids mainly separated by hydrophobic residues. S5–S6 segments along with the intervening pore loop contribute to the pore domains. S5 is connected to S6 by an extracellular helix, followed by the pore helix (PH) and the K^+ selective filter (SF) (Jiang et al. 2005). The SF of the hERG channel adopts a unique signature sequence of Ser-Val-Gly-Phe-Gly (Doyle et al. 1998). It has been supposed that below the SF the pore widens to form a water-filled central cavity that is lined by residues from the S6 helices (Perry et al. 2010). However, recently solved cryo-EM structure of the hERG channel in the open state reveals that four deep cylindrical hydrophobic pockets below the SF extend out from the central pore cavity (Fig. 1a) (Wang and MacKinnon 2017). These pockets exclusively exist in hERG channel since the S6 inner helix of hERG is displaced to create a separation between the PH and S6 helix (Wang and MacKinnon 2017).

hERG channel has a unique kinetic behavior that is characterized by slow deactivation but very fast, voltage-dependent inactivation (Vandenberg et al. 2004). This unusual combination of kinetics gives rise to an apparent inward rectification that is crucial for maintaining a prolonged plateau phase of the cardiac AP. The channel opens following membrane depolarization as a result of its VSD's response to the voltage; however, the channel almost immediately inactivates, limiting K^+ passage until the start of the repolarization phase of the AP (due to the rapid recovery from inactivation). In addition, hERG deactivates very slowly so that the outward K^+ current is passed even as the membrane potential returns toward the resting potential (Fig. 1b, c). Therefore, the unique kinetics makes the hERG current ideally suited for determining the duration of the plateau phase of the AP (Smith et al. 1996; Sanguinetti and Tristani-Firouzi 2006). Maintenance of plateau is crucial for ensuring sufficient time for calcium release from the sarcoplasmic reticulum to enable cardiac contraction. The gating kinetics of hERG also enables the channel to generate rapid transient currents late in AP repolarization/early diastole, to protect against arrhythmogenic premature depolarizations.

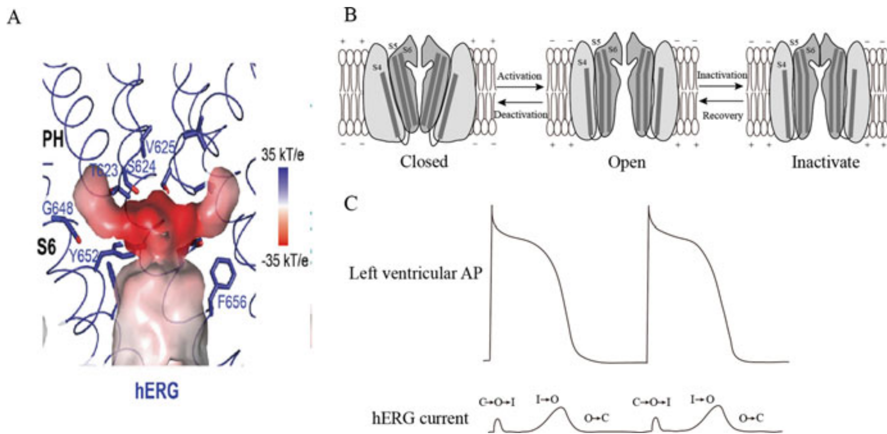

Fig. 1 The structure and gating of hERG channel. (**a**) The structure of pore cavity; adopted from (Wang and MacKinnon 2017) with permission. The central cavity has an atypically small central volume surrounded by four deep hydrophobic pockets. Internal molecular surface around the central cavity is represented as translucent surface colored by electrostatic potential according to the scale shown. Residues related to drug binding are shown as sticks on the otherwise ribbon representation of the channel. (**b**) hERG channel exists in closed, open, or inactivated states; transitions between these states are voltage dependent. (**c**) hERG current response (bottom) to the AP voltage waveform (top). hERG channel opens following membrane depolarization and then rapidly inactivates. During repolarization of the AP waveform, the current increases due to the recovery from inactivation and then slowly decreases again as the electrochemical gradient for K^+ efflux decreases

3 Mechanisms of Arrhythmias

Cardiac arrhythmias are commonly believed to arise primarily from abnormal automaticity, reentrant excitation, or the combination of both. Abnormal automaticity may occur as a result of enhanced automaticity or triggered activity (Wit 1990). The triggered activity and reentrant excitation are highly associated with hERG dysfunction-induced tachycardial ventricular arrhythmias. It is generally accepted that tachyarrhythmic events are obligated depending on two phenomena: a triggering event for initiation and a reentry substrate for sustainability (Schmitt et al. 2014). Triggered activity results from the premature activation of cardiac tissues by afterdepolarizations, which are oscillations in membrane potential that follow the primary depolarization phase (0) of an AP. If afterdepolarizations develop before full repolarization, corresponding to phase 2 or phase 3 of the cardiac AP, they are classified as early afterdepolarizations (EADs) and those originating from phase 4 of AP are classified as delayed afterdepolarizations (DADs) (Fig. 2a). EADs are usually but not exclusively associated with prolonged action potential durations (APD). It is generally considered that EADs occur primarily due to the reactivation of the voltage-gated $Ca_V1.2$ channels (L-type Ca^{2+} channels) (January and Riddle 1989). If the change in membrane potential brought about by the EAD is large enough to

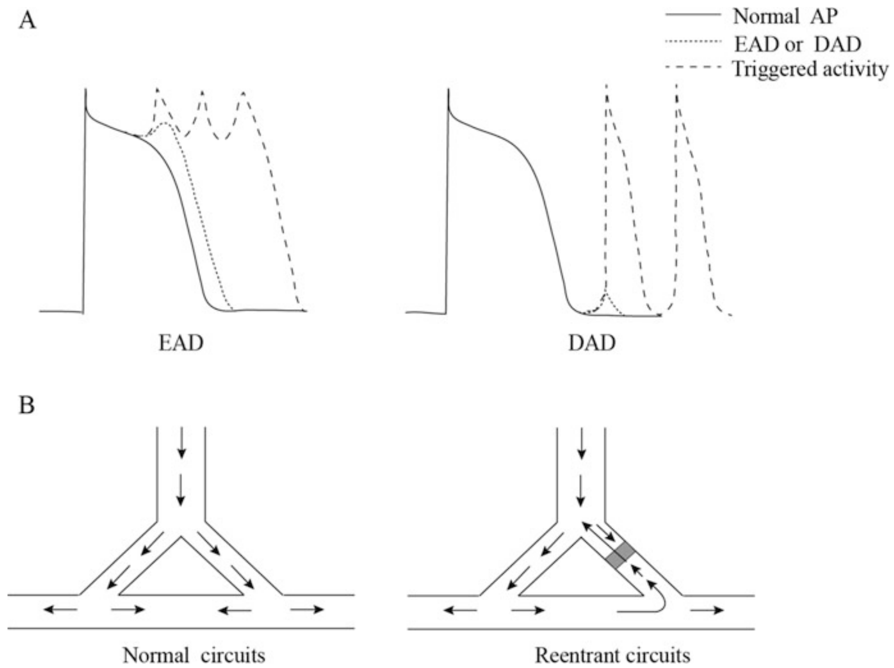

Fig. 2 The mechanisms of arrhythmias. (**a**) The afterdepolarizations developing before full repolarization, corresponding to phase 2 or phase 3 of the cardiac AP are classified as EADs (left,⋯⋯), and those originating from phase 4 of AP are classified as DADs (right,⋯⋯). When afterdepolarizations reach the threshold potential, a new AP is generated, leading to the triggered activity (-----). (**b**) Propagation of normal AP (left) and conditions for a reentrant excitation (right). Under normal conditions, the electrical signals travel down each branch of Purkinje fiber with equal velocity, and the signals will not progress if the two branches are connected. However, if one branch exhibits a unidirectional block, the electrical signal will travel down only one branch and may back-propagate until the point of blocking. If a retrogradely progressing impulse encounters excitable tissue, a reentry is set up

reach the threshold potential for initiation of APs, it will cause triggered activity (Fig. 2a). EADs and their resulting triggered activity are thought to underlie the arrhythmogenesis observed in LQTS (Maruyama et al. 2011). DADs usually occur under conditions of intracellular calcium overload and involve spontaneous release of calcium from the sarcoplasmic reticulum.

In order for sustained arrhythmias to occur, the triggering events must subsequently initiate a self-sustained episode of APD propagation, which is known as reentry-based arrhythmia (where reentry denotes an ongoing loop of unintended electrical signaling). A normally-propagating AP usually encounters neighboring tissue with equal conducting velocity and completely extinguish (Fig. 2b left). If an impulse is blocked in a specific area of the tissue but not elsewhere and the retrograde conduction is still possible, a unidirectional blocking is said to have occurred. If a retrogradely conducting impulse encounters excitable tissue, a reentry

is being set up (Fig. 2b right). Such electrophysiological blocks may result from an anatomical or functional obstacle under pathological conditions such as myocardial infarction or inflammation or altered electrophysiologic properties due to electrolyte imbalance or ischemia. Another important factor forming arrhythmic substrates is electrophysiological heterogeneity of the myocardium. The APD diverges in different parts of the myocardium, and there is a significant heterogeneity among cardiac cells along several axes including the transmural, left-right, and apicobasal axes (Boukens et al. 2009). The dispersion is increased in the conditions with inherited ion channelopathies and after unintended inhibition of I_{Kr} by cardiac and non-cardiac drugs (Antzelevitch 2007, 2008). This amplification of spatial dispersion of repolarization can form substrates for reentry loops and thus contribute to life-threatening arrhythmias (Antzelevitch 2007; Keating and Sanguinetti 2001).

4 hERG Inhibitors

4.1 hERG Inhibitors as Antiarrhythmic Agents

Class III antiarrhythmic agents include nonselective K^+ channel blockers ambasilide, amiodarone, and dronedarone and selective I_{Kr} blockers dofetilide, ibutilide, and sotalol (Lei et al. 2018). The supposed mechanism of antiarrhythmic effects of these compounds is the inhibition of reentry-based arrhythmias through prolongation of the effective refractory period (ERP). However, inhibition of I_{Kr} by these compounds has also been found to be associated with an increased risk of arrhythmias and sudden cardiac death (Vandenberg et al. 2001). The proarrhythmic effect of class III compounds results from excessive prolongation of APD, especially an extended and slowly decaying phase 3-repolarization (triangulation), which could promote reactivation of L-type Ca^{2+} channels and, thus, lead to EADs. According to the aforementioned arrhythmogenic mechanisms, increased dispersion of repolarizations form reentry substrates can, in turn, result in TdP, which may ultimately degenerate to ventricular fibrillation.

4.2 hERG Inhibition by Structurally Diverse Drugs

In 1922, syncope and sudden death were firstly reported in patients treated with the quinidine (Levy 1922). These phenomena were further revealed in 1964, when Selzer and Wray (1964) observed TdP on electrocardiograms from patients with quinidine-related syncope, which was resulted from prolongation of cardiac repolarization due to hERG channel blockage. Since then, more and more drugs with miscellaneous structures are discovered to block hERG channel and, thus, carry the TdP risk. Antiarrhythmic, antihistamine, antimicrobial, antipsychotic, and antidepressant drugs are important classes associated with proarrhythmic risk (Rampe and Brown 2013). Hitherto, several drugs have been withdrawn from the market or given strict limitation for use because of TdP risk, including terfenadine, lidoflazine,

astemizole, sertindole, levomethadyl, droperidol, cisapride, and grepafloxacin (Table 1). A database is available for drugs with the risk of TdP, which is categorized into three classes: drugs with known risk of TdP, possible risk of TdP, and conditional risk of TdP. Drugs with known risk of TdP related to hERG channel inhibition are listed in Table 1. Updated information about drug-associated TdP risk can be found at www.crediblemeds.org.

4.3 Molecular Basis Underlying hERG Channel Inhibition

The question of why the hERG channel is so susceptible to "nonspecific" block by such a wide variety of medications has attracted intense interest. Much effort has been made to explore the structural basis underlying this unusual susceptibility to inhibition, with approaches ranging from electrophysiology to, protein structure solution and *in silico* modeling. It is generally considered that there are at least two important structural features of hERG channel that are responsible for the above property. Firstly, many drugs bind to hERG channel by being trapped in its inner cavity, which appears to be much larger than in any other voltage-gated K^+ channel. Thus, the large inner cavity of hERG channel can accommodate and trap large molecules that other K^+ channels cannot trap (Mitcheson et al. 2000). Recently, the cryo-EM structure of hERG has been solved (Wang and MacKinnon 2017), it provides a valuable insight into the channel structure with regard to the drug binding. It has been demonstrated that there are four unique elongated, relatively hydrophobic pockets that extend from the central cavity (Wang and MacKinnon 2017) (Fig. 1a). Drugs are proposed to occupy the center of the cavity and insert a functional group into the hydrophic pockets. The central cavity of the channel in the region just below the SF is slightly narrower than that seen in *Shaker-like* voltage-gated K^+ channel structures. As a consequence, there is a greater negative electrostatic potential in this region of the cavity (Vandenberg et al. 2017), which attracts cations (e.g., metal ions or positively charged drugs) to form a more stable structure. Secondly, it is believed that a number of aromatic residues in a specific hERG channel region can form binding sites for inhibitory drugs. The electrons of the aromatic ring may form π-cation or π-π interactions with the drug molecule *via* charged nitrogen or aromatic ring, respectively (Fernandez et al. 2004; Stansfeld et al. 2007). Mutagenesis screening has demonstrated that residues on the S6 helix (Y652, F656, G648) and residues at the base of the SF (T623, S624, and V625) are critical to binding for a range of hERG blockers (Kamiya et al. 2006; Lees-Miller et al. 2000; Mitcheson et al. 2000; Perry et al. 2004). Among these, the two aromatic residues on the S6 helices (Y652, F656) are highly conserved in hERG channel orthologs, but not in other voltage-dependent K^+ channels (Shealy et al. 2003). Substantial evidence has shown that channel blockage by almost all hERG blocking drugs tested is dramatically attenuated by mutations of one or both of these two key residues (Y652 and F656) that form much of the lining of the K^+ conductance pathway. In addition, *in silico* hERG blocking studies have also demonstrated that Y652 and F656 in the hERG S6 domain play critical roles in drug binding (Hyang-Ae et al. 2018). These

Table 1 Drugs with a known risk of TdP due to hERG inhibition

Drugs	Drug class	References
Amiodarone	Antiarrhythmic	(Kamiya et al. 2001; Kiehn et al. 1999)
Arsenic trioxide[a]	Anticancer	(Ficker et al. 2004)
Astemizole[b]	Antihistamine	(Suessbrich et al. 1996; Zhou et al. 1999)
Azithromycin	Antibiotic	(Yang et al. 2017; Zhi et al. 2015)
Bepridil[b]	Antianginal	(Chouabe et al. 1998, 2000)
Chloroquine	Antimalarial	(Sánchez-Chapula et al. 2002; Traebert et al. 2004)
Chlorpromazine	Antipsychotic/ antiemetic	(Lee et al. 2004; Thomas et al. 2003b)
Ciprofloxacin	Antibiotic	(Bischoff et al. 2000; Kang et al. 2001)
Cisapride[b]	GI stimulant	(Mohammad et al. 1997; Rampe et al. 1997)
Citalopram[a]	Antidepressant, SSRI	(Chae et al. 2014; Witchel et al. 2002)
Clarithromycin	Antibiotic	(Stanat et al. 2003; Volberg et al. 2002)
Cocaine	Local anesthetic	(Guo et al. 2006; Zhang et al. 2001)
Disopyramide	Antiarrhythmic	(Paul et al. 2001; Yang et al. 2001)
Dofetilide	Antiarrhythmic	(Kiehn et al. 1995; Yang et al. 2001)
Domperidone	Antiemetic	(Claassen and Zünkler 2005; Drolet et al. 2000)
Donepezil[a]	Cholinesterase inhibitor	(Chae et al. 2015)
Dronedarone	Antiarrhythmic	(Ridley et al. 2004; Thomas et al. 2003a)
Droperidol	Antipsychotic/ antiemetic	(Drolet et al. 1999; Luo et al. 2008)
Erythromycin	Antibiotic	(Duncan et al. 2006; Stanat et al. 2003)
Escitalopram[a]	Antidepressant, SSRI	(Chae et al. 2014)
Flecainide	Antiarrhythmic	(Paul et al. 2002)
Fluconazole[a]	Antifungal	(Han et al. 2011)
Gatifloxacin[b]	Antibiotic	(Kang et al. 2001)
Grepafloxacin[b]	Antibiotic	(Bischoff et al. 2000; Kang et al. 2001)
Halofantrine	Antimalarial	(Tie et al. 2000; Traebert et al. 2004)
Haloperidol	Antipsychotic	(Shuba et al. 2001; Suessbrich et al. 1997)
Ibogaine	Psychedelic	(Koenig et al. 2013; Thurner et al. 2014)
Ibutilide	Antiarrhythmic	(Kodirov et al. 2019; Yang et al. 2001)
Levofloxacin	Antibiotic	(Kang et al. 2001)
Levomethadyl acetate[b]	Opioid agonist	(Katchman et al. 2002)
Mesoridazine[b]	Antipsychotic	(Su et al. 2004)
Methadone	Opioid agonist	(Katchman et al. 2002)
Moxifloxacin	Antibiotic	(Bischoff et al. 2000; Kang et al. 2001)
Nifekalant	Antiarrhythmic	(Kushida et al. 2002)
Ondansetron	Antiemetic	(Kuryshev et al. 2000)
Papaverine HCl (Intracoronary)	Vasodilator, coronary	(Kim et al. 2007, 2008)

(continued)

Table 1 (continued)

Drugs	Drug class	References
Pentamidine[a]	Antifungal	(Kuryshev et al. 2005; Tanaka et al. 2014)
Pimozide	Antipsychotic	(Kang et al. 2000)
Probucol[a,b]	Antilipemic	(Guo et al. 2007, 2011)
Procainamide	Antiarrhythmic	(Yang et al. 2001)
Propofol	Anesthetic, general	(Han et al. 2016)
Quinidine	Antiarrhythmic	(Sănchez-Chapula et al. 2003; Yang et al. 2001)
Roxithromycin[a]	Antibiotic	(Han et al. 2013; Volberg et al. 2002)
Sertindole[b]	Antipsychotic	(Rampe et al. 1998)
Sevoflurane	Anesthetic, general	(Yamada et al. 2006)
Sotalol	Antiarrhythmic	(Numaguchi et al. 2000; Sanguinetti and Jurkiewicz 1990)
Sparfloxacin[b]	Antibiotic	(Bischoff et al. 2000; Kang et al. 2001)
Sulpiride	Antipsychotic, atypical	(Lee et al. 2009)
Terfenadine[b]	Antihistamine	(Suessbrich et al. 1996; Tanaka et al. 2014)
Terodiline	Muscle relaxant	(Martin et al. 2006)
Thioridazine	Antipsychotic	(Kim and Kim 2005; Milnes et al. 2006)
Vandetanib	Anticancer	(Lee et al. 2018)

[a]Drug with effect of trafficking inhibition
[b]Drug withdrawn from market by FDA. Data acquired from www.crediblemeds.org in May, 2020

two aromatic residues in each subunit were originally proposed to face into the inner cavity so as to provide a total of eight binding sites for drugs (Mitcheson et al. 2000). However, recent cryo-EM structure of hERG channel in open state revealed that Y652 projects towards K^+ permeation pathway, while F656 side chains projects away from the permeation pathway towards the outer PH (Fig. 1a). This structure is not consistent with the original hypothesis that drugs directly bind to F656 within the permeation pathway. The molecular basis for this discrepancy is not yet fully understood. One possibility is that inactivation in hERG is associated with repositioning of Y652 and (especially) F656 side chains into a configuration that promotes interaction with blockers in the pore since drugs prefer to bind to the hERG channel in its inactivated state (Chen et al. 2002). This might involve a small clockwise rotation of the inner S6 helix containing these side chains (Chen et al. 2002; Helliwell et al. 2018). Comprehensive reviews with the detailed information about molecular basis of hERG drug binding can be found in the references (Butler et al. 2019; Dickson et al. 2020; Helliwell et al. 2018; Vandenberg et al. 2017; Wacker et al. 2017; Wang and MacKinnon 2017).

In addition to the direct inhibition of channel activity, forward trafficking impairment can reduce hERG current through a reduction in the number of hERG channels on cell membrane. Experiments indicate that arsenic trioxide (Ficker et al. 2004), pentamidine (Kuryshev et al. 2005), and probucol (Guo et al. 2007) disrupt hERG trafficking at concentrations known to cause QT prolongation and arrhythmia

without direct channel block. Some other drugs such as fluoxetine and ketoconazole both can acutely block hERG channel and reduce hERG plasma membrane protein abundance following long-term exposure by inhibiting trafficking (Rajamani et al. 2006; Takemasa et al. 2008). It is important to consider impaired trafficking as an alternative mechanism for drug-induced QT prolongation, as conventional compound screening methods for hERG block liability may not detect reductions in channel abundance.

4.4 Methodology of hERG Assays

Since hERG channel plays an important role in cardiac repolarization and is susceptible to inhibition by a wide variety of compounds, evaluation of the potential hERG blocking effect of new compounds for identifying potential risk of proarrhythmic side effects is a necessary step in a drug discovery process. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) adopted a guideline S7B putting forward requirements in assessing hERG blocking of new drugs for the cardiac safety in 2005.

A variety of technologies have been applied to evaluate effects of hERG channel blocking based on multiple test systems including heterologous hERG expression in Xenopus oocyte and mammalian cells such as HEK293 cells and CHO cells, and native cardiomyocytes with I_{Kr} current. Because the cardiomyocytes of adult mice and rats heart lack the I_{Kr} current component, native cardiomyocytes for testing are commonly derived from the hearts of larger animals such as guinea pigs, rabbits, and dogs. Evaluation technologies include direct electrophysiological measurement (i.e., patch clamp), and indirect non-electrophysiological measurements such as competitive radioligand binding assays, ion flux assays, fluorescence-based assays, and *in silico* modeling.

Patch clamp technique remains a gold standard to directly assess hERG blocking liability of compounds (Hancox et al. 2008). It provides accurate and physiologically relevant data of ion channel function at the single cell or single channel level. However, traditional manual patch clamp has been limited in drug screening due to low throughput and a requirement for highly skilled operators. Recently developed automated patch clamp approach, which offers high-throughput electrophysiological data acquisition, has transformed the situation (Guo and Guthrie 2005; Jones et al. 2009). At present, both manual patch clamp and automated patch clamp are widely used in evaluation of hERG safety (Danker and Möller 2014; Lindqvist 2019). Non-electrophysiological measurements are also widely used, these assess the potency of drugs for hERG blocking by measuring the hERG channel related indicators. The competitive radioligand binding assays determine displacement of specific radiolabeled hERG ligands such as [^{3}H]dofetilide (Diaz et al. 2004; Finlayson et al. 2001a, b), [^{3}H]astemizole (Chiu et al. 2004), [^{35}S]-MK-499 (Raab et al. 2006), and [^{125}I]-BeKm1 (Angelo et al. 2003) to reflect the binding affinity of test drugs. The ion flux-based assays (often in combination with fluorescence-based

approaches) measure the amount of ions such as Rubidium (Rb^+) (Terstappen 1999) and Thallium (Tl^+) (Titus et al. 2009; Weaver et al. 2004) permeating through the hERG channel and thus indirectly reflect the alterations of hERG function under the action of drugs. In recent years, *in silico* models of hERG channel were developed for predicting the action of hERG modulators. *In silico* models are based on structural properties of the hERG channel and incorporate the information of channel gating and ligand binding kinetics. The aim of such modeling is to characterize the interactions of compounds with the hERG channel by computer simulations (Lee et al. 2016; Pearlstein et al. 2016; Zhang and Hancox 2004). However, the electrophysiological measurements remain necessary to confirm data obtained by such modeling.

The potency of compounds for producing hERG inhibition, usually indicated by the compound's IC_{50} (concentration of half-maximal inhibition), can be normalized to the clinically relevant concentrations of the given compound, such as $C_{max,free}$ (free plasma concentration) to calculate the safety margin, as proposed by the S7B guideline. According to relevant studies, the closer the hERG IC_{50} value is to the $C_{max,free}$ the higher is the risk of QT interval prolongation (Redfern et al. 2003; van Noord et al. 2011). A 30-fold margin between $C_{max,free}$ and hERG IC_{50} has been considered as a cardiac safety value in many cases (Redfern et al. 2003; van Noord et al. 2011). However, it is also recognized that an increase in the margin should be considered, especially for drug candidates aimed for non-debilitating diseases (Redfern et al. 2003).

However, due to the lack of standardization for measuring hERG modulator potency, there are often significant differences in measured IC_{50} values reported by different laboratories for the same compounds. For instance, the difference in IC_{50} of cisapride reported by different laboratories exceeds 60-fold (Potet et al. 2001; Rezazadeh et al. 2004). The essential factors that contribute to such variability generally include differences in test systems and recording conditions such as temperature and voltage protocols.

Using different test systems, such as native cardiac myocytes and cell lines heterologously expressing hERG can lead to significant discrepancy of IC_{50} values. As much as 50-fold difference of E-4031 IC_{50} has been observed between native cardiac myocytes (Sanguinetti and Jurkiewicz 1990) and transfected cells (Zhou et al. 1998). This discrepancy may result from the differences in the composition of hERG channel. In the native cardiomyocytes, in addition to the dominant hERG1a isoform, the hERG1b isoform is also expressed (although at much lower level) and can contribute to the composition of heteromeric channel (McNally et al. 2017). Indeed, a study has shown that a homomeric hERG1a channel expressed in HEK293 cells is blocked by E-4031 more rapidly than with a heteromeric channel containing both hERG1a and hERG1b (Sale et al. 2008). A similar trend has been found for dofetilide (Abi-Gerges et al. 2011).

Additional complications arise from the state-dependent binding of some compounds to the hERG. Substantial evidence indicates that different hERG blockers have a high-affinity binding to the activated or inactivated channel (Stork et al. 2007; Walker et al. 1999). The channel state can be modulated by temperature

and voltage protocols including voltage pattern, duration, and pulse frequency (Lee et al. 2019; Stork et al. 2007). Thus, it is not difficult to understand why there are significant differences in measured IC_{50} values under distinct temperature and voltage protocols. In addition, temperature and voltage protocols have an influence on drug binding kinetics and trapping (Kirsch et al. 2004; Stork et al. 2007). These factors also lead to discrepancies in the reported potency parameters.

4.5 A New CiPA Paradigm to Evaluate Drug-Induced TdP

Although no approved drugs have been withdrawn from the market because of the TdP risk since the ICH S7B Guideline was implemented (Sager et al. 2014), the hERG safety remains a necessary phase in drug discovery. Yet, limitations of only assessing hERG blockage have been recognized. The cardiac AP is coordinated by multiple ion currents and requires relative balance between inward and outward currents. It is therefore insufficient to focus on a single component in predicting the risk of delayed repolarization and TdP. For example, verapamil has been shown to inhibit hERG current with high potency (Zhang et al. 1999), but it does not lead to QT interval prolongation and does not increase the TdP risk because of the concomitant inhibition on inward I_{CaL} (Winters et al. 1985). A recent study based on 30 drugs of different risk categories (high, intermediate, and low) has shown that blocking inward currents such as sodium and calcium current may reduce proarrhythmic effect of hERG current inhibition (Crumb et al. 2016). Thus, assessing hERG blockage alone carries a risk for false-positive predictions and leads to potentially valuable new compounds being discarded early in drug discovery. A study has indicated that as many as 60% of new molecular entities developed as potential therapeutic agents are abandoned early due to hERG inhibition (Ponti 2008). Therefore, a new parardigm, a Comprehensive Invitro Proarrhythmia Assay (CiPA) has been proposed in the field of cardiac safety; CiPA presents a more comprehensive approach to predicting proarrhythmic risk (Sager et al. 2014).

There are three preclinical components in CiPA paradigm: (1) drug effects on multiple human cardiac currents; (2) *in silico* reconstruction of human ventricular electrophysiology, and (3) *in vitro* effects on human stem-cell derived ventricular myocytes. Specific study groups have been established to refine the approaches and benchmarks within each of these components.

In CiPA paradigm, hERG blocking is no longer the unique indicator; instead, a more comprehensive *in vitro* set of ion current assays is used to explore the effects of drugs on multiple potassium, sodium, and calcium currents. A recent study has shown that, under the premise of evaluation of hERG, incorporating $Na_V1.5$ or $Ca_V1.2$ in particularly into the evaluation system has significantly improved the TdP predictability (Kramer et al. 2013). The ion channel working group of CiPA has developed a series of protocols to test the effects of compounds on the main cardiac ion channels including hERG, L-type calcium, and fast and late inward sodium currents, hoping to provide standardized protocols to be used in different patch clamp facilities of the academic and industrial research institutions (Fermini et al.

2016; Huang et al. 2017; Windley et al. 2017). In the next step, *in silico* reconstruction of ventricular APs assesses the effects of compounds more intimately on the basis of electrophysiological data. Finally, cadiomyocytes such as human induced pluripotent stem cell-derived cardiac myocytes (hiPSC-CMs) would be used to provide an assessment of the integrated electrophysiological response to a drug (Sager et al. 2014; Wallis et al. 2018). The updated information about the progress of CiPA groups is available at www.cipaproject.org. Hopefully, the CiPA paradigm can provide more precise and comprehensive information for assessment of hERG inhibition to predict the risk of drug-induced arrhythmia.

5 hERG Activators

In contrast to numerous hERG channel blockers, some compounds have been discovered to increase hERG channel currents during the course of screening for hERG channel-blocking activity early in preclinical safety evaluation (Grunnet et al. 2008). Thus, Kang and colleagues reported the first synthetic activator of hERG channel, RPR260243 (Kang et al. 2005). Since then several other hERG activators have been identified, including PD118057 (Zhou et al. 2005), NS1643 (Casis et al. 2006; Hansen et al. 2006a), NS3623 (Hansen et al. 2006b), Mallotoxin (Zeng et al. 2006), PD307243 (Gordon et al. 2008; Xu et al. 2008), A935142 (Su et al. 2009), ICA-105574 (Gerlach et al. 2010), KB130015 (Gessner et al. 2010), etc. These compounds shorten cardiac APD and have been proposed as a new therapeutic approach for the treatment of acquired or congenital LQTS (reviewed in (Sanguinetti 2014; Szabó et al. 2011; Vandenberg et al. 2012; Zhou et al. 2011)).

5.1 Mechanisms of Action of hERG Channel Activators

Different to hERG blockers that simply block K^+ conduction and have little influence on channel gating, hERG activators primarily exert their effects by modulating channel gating. Four distinct mechanisms have been described: (1) slowing the rate of channel deactivation; (2) attenuation of C-type inactivation; (3) negative shift of voltage dependence of activation; (4) increase in channel open probability (Sanguinetti 2014) (Fig. 3). Accordingly, depending on the predominant mechanism of action, hERG activators can be categorized in four types (although most hERG activators have multiple mechanisms of action). Here, we will give a brief review on the gating modulation by several known activators. More detailed information on these mechanisms can be found in several previous reviews (Perry et al. 2010; Sanguinetti 2014; Szabó et al. 2011; Zhou et al. 2011). The chemical structures of major hERG activators are shown in Fig. 4.

5.1.1 Slowing the Deactivation

RPR260243 is the first compound designed as a type 1 hERG channel activator (Kang et al. 2005). This small molecule enhances current by attenuating inactivation

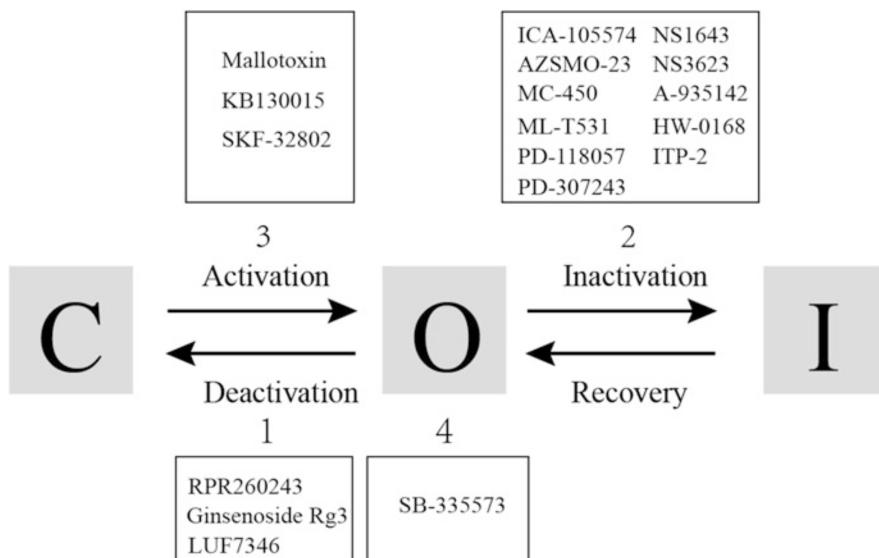

Fig. 3 The action of hERG channel activators. hERG activators primarily exert their effects by modulating channel gating. There are four distinct mechanisms including slowing of channel deactivation (1), attenuation of C-type inactivation (2), negative shift of voltage dependence of activation (3), and increase in channel open probability (4). Known hERG activators are assigned to types 1-4, according to the predominant mechanism of action

and severely slowing the rate of channel deactivation (Kang et al. 2005; Perry et al. 2007). Another compound, Ginsenoside Rg3, an alkaloid isolated from the root of *Panax ginseng* plants, increases current magnitude primarily by slowing the rate of hERG deactivation (Choi et al. 2011). More recently, compound LUF7346 has been identified as a type 1 hERG channel activator, which increases hERG current by slowing deactivation and positively shifting voltage dependence of inactivation (Sala et al. 2016).

Scanning mutagenesis has identified the putative binding site for RPR260243, which is located near the cytoplasmic ends of the S5 and S6 helices of the hERG subunit, a region of the channel that is important for activation and deactivation. Hence, it is proposed that binding of RPR260243 to a single subunit may directly constrain movement of the S6 domains to slow the rate of channel closure (Perry et al. 2007).

5.1.2 Attenuation of C-Type Inactivation

As mentioned, one of the most important gating features of hERG channel is its fast C-type inactivation. Attenuation of C-type inactivation is produced by some of the hERG channel activators, an effect resulting in an enhancement of hERG current. Up to now, more than ten compounds such as PD118057 (Zhou et al. 2005), PD307243 (Gordon et al. 2008), NS1643 (Casis et al. 2006), NS3623 (Hansen et al. 2006b), A-935142 (Su et al. 2009), ICA-105574 (Gerlach et al. 2010), ML-T531 (Zhang

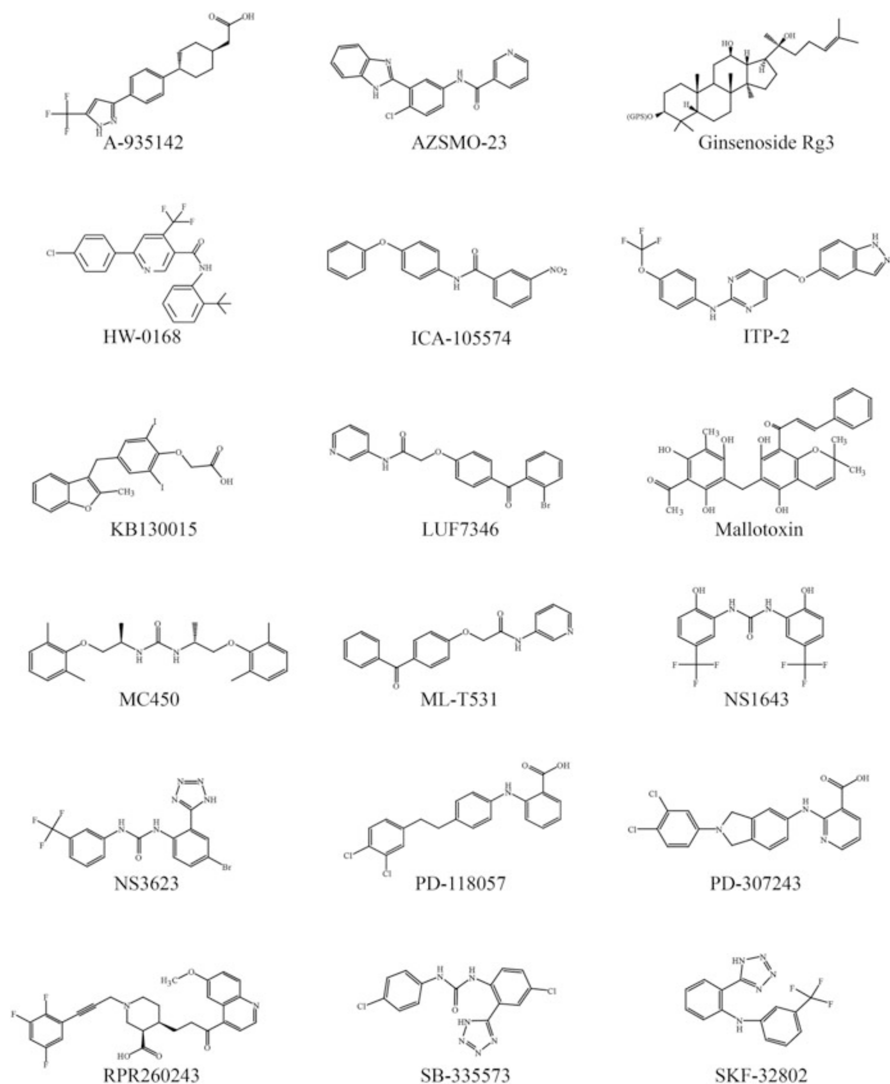

Fig. 4 Chemical structures of major hERG channel activators

et al. 2012), AZSMO-23 (Mannikko et al. 2015), ITP-2 (Sale et al. 2017), MC-450 (Gualdani et al. 2017), and HW-0168 (Dong et al. 2019) have been identified to enhance hERG current primarily through attenuating the channel inactivation and are thus classified as type 2 activators (Perry et al. 2010). However, most of those activators may have multiple mechanisms of action. The mechanistic and structural basis underlying the fast inactivation of hERG channel is not fully understood. It is believed to be caused by a subtle voltage-dependent conformational changes in the SF of the outer pore domain (for reviews, see Ref. Vandenberg et al. 2012).

Experimental evidence has shown that the binding sites of many type 2 activators are located closer to the SF (Garg et al. 2011; Gerlach et al. 2010; Perry et al. 2009). Scanning mutagenesis combined with molecular modeling studies have revealed that PD118057 interacts with residues located in the PH of one hERG subunit and the N-terminal half of the S6 helix in an adjacent subunit to attenuate inactivation (Perry et al. 2009). Similarly, the residues interacting with ICA-105574, another potent type 2 activator (Gerlach et al. 2010), are located in the PH and the base of the SF and S6 segments (Garg et al. 2011). A recent study has proposed a common mechanism to prevent C-type inactivation by a group of negatively charged activators such as PD-118057 (Schewe et al. 2019). This type of activators may directly stabilize the SF in its active state through binding to similar sites below the SF (Schewe et al. 2019). In line with this hypothesis, a molecular dynamics simulation has demonstrated that ICA-105574 increases the stability of the SF to attenuate channel inactivation (Zangerl-Plessl et al. 2020). However, whether other type 2 activators with distinct chemical structures share the same molecular mechanism remains uncertain.

5.1.3 Negative Shift of Voltage Dependence of Activation

Previous experimental findings indicate that both Mallotoxin and KB130015 increase hERG current amplitude primarily by causing a hyperpolarizing shift in the voltage dependence of channel activation (Zeng et al. 2006; Gessner et al. 2010). Mallotoxin also accelerates the rate of activation and slows the rate of deactivation (Zeng et al. 2006). KB130015 is a derivative of the hERG blocker, amiodarone, and presumably binds to the hERG pore from the cytosolic side and functionally competes with amiodarone (Gessner et al. 2010). SKF-32802, a structural analog of NS3623, induces a leftward shift in the voltage dependence of activation. The above compounds are identified as the type 3 activators (Donovan et al. 2018).

5.1.4 Increase in Channel Open Probability

Similar to SKF-32802, SB-335573 is also a structural analog of NS3623. However, it enhances hERG current through increasing open probability without affecting the voltage dependence of activation and, thus, identified as a type 4 activator (Donovan et al. 2018). In addition, PD-118057 has been reported to increase single hERG channel open probability (Perry et al. 2009).

5.2 Potential Antiarrhythmic Effect of hERG Channel Activators

Several hERG activators have been tested for their antiarrhythmic effectiveness in inherited or drug-induced acquired LQTS. Thirteen subtypes of inherited LQTS have been identified, with the most prevalent forms being LQTS1, 2, and 3 (Schwartz et al. 2012). The underlying channelopathies are loss-of-function mutations in I_{Ks} (type 1) and in I_{Kr} (type 2) and increased sustained I_{Na} current (type 3). Theoretically, the LQTS phenotype could be rescued by the compensatory effect of hERG channel activators if I_{Kr} current is not completely lost. Experimental evidence

obtained in cardiac myocytes, especially in hiPSC-CMs derived from LQTS patients and in transgenic animals, supports this notion. A study has demonstrated that NS1643 significantly shortens APD and QT interval in a rabbit model of inherited LQTS1 (Bentzen et al. 2011). Type 2 activator ML-T531 normalizes the prolonged APD by selectively enhancing I_{Kr} in hiPSC-CMs derived from LQTS1 patient (Zhang et al. 2012). NS1643 and ICA-105574 effectively restore hERG current from heterozygous LQTS2 mutant channels in heterologous expression systems (Huo et al. 2017; Perry et al. 2020). Several activators, including NS1643, ICA-105574, and LUF-7346, have been shown to reverse the prolonged repolarization in hiPSC-CMs derived from LQTS2 patients carrying different mutations (Duncan et al. 2017; Perry et al. 2020; Sala et al. 2016). In addition, both NS3623 and Mallotoxin show the antiarrhythmic potential in a cellular model of LQTS3 (Diness et al. 2009).

Many hERG activators with different gating modulation mechanisms have been demonstrated to counteract the inhibition by hERG blockers either in heterologous expression systems or in native cardiac myocytes (review, Ref. (Szabó et al. 2011)). However, only few of those activators have been tested *in vivo* or in intact hearts for their effectiveness of suppressing drug-induced arrhythmias. An experiment has demonstrated that *in vivo* administration of NS3623 results in shortening of the QT interval as well as reversal of a pharmacologically induced QT prolongation in both anesthetized and conscious guinea pigs (Hansen et al. 2008). NS1643 completely suppresses arrhythmic activity caused by I_{Kr} inhibitor dofetilide in the *in vivo* rabbit models of TdP (Diness et al. 2008). ICA-105574 effectively prevents ventricular arrhythmias caused by I_{Kr} or I_{Ks} inhibitors in intact guinea-pig hearts (Meng et al. 2013). Recent experiments demonstrates that LUF7244 and RPR260243 counteract dofetilide-induced arrhythmias in a chronic atrioventricular block model in dogs (Qile et al. 2019) and in whole organ zebrafish hearts (Shi et al. 2020), respectively. These findings support the notion that hERG activators may provide an effective antiarrhythmic approach in drug-induced, disease-induced, or gene mutation-linked LQTS.

5.3 Proarrhythmic Risk of hERG Channel Activators

The fact that congenital short QT syndromes (SQT) (Crotti et al. 2010) may lead to susceptibility to arrhythmias raises concerns that QT-shortening drugs could also lead to arrhythmias. Several reports have revealed the potential proarrhythmic risk of some hERG activators including mallotoxin, NS1643, ICA-105574, and PD-118057 in experimental and *in silico* models (Bentzen et al. 2011; Lu et al. 2008; Peitersen et al. 2008; Schewe et al. 2019) and, thus, those hERG activators have been used to create drug-induced SQT models. The arrhythmogenesis of these activators may result from a decrease of ERP and an increase of the transmural dispersion of repolarization (TDR). Amplification of the spatial dispersion of repolarization in the form of TDR is the basis for the development of life-threatening ventricular arrhythmias (Antzelevitch 2007). In addition, ICA-105574 causes temporal

redistribution of the peak I_{Kr} to much earlier in the plateau phase of the AP and, thus, results in early repolarization (Perry et al. 2020; Qiu et al. 2019), which, in turn, my result in the development of phase 2 reentry and ventricular tachycardia/ventricular fibrillation.

6 Conclusion

The shape of the cardiac AP depends on a fine balance between various depolarizing and repolarizing ionic currents. The unique gating kinetic properties of hERG channel make it ideal for determining the morphology and duration of the cardiac AP repolarization. Consequently, alterations of hERG channel function by inhibitors or activators may result in either prolongation or shortening of APD, which can counteract abnormal electroactivity under specific pathological condition. However, unintended disturbance or overcorrection of hERG channel function may result in arrhythmogenesis. Thus, hERG channel becomes an important pharmacological and safety target for antiarrhythmic/proarrhythmic actions of drugs.

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