

Drugs, hERG and sudden death

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Abstract

Early recognition of potential QT/TdP liability is now an essential component of the drug discovery/drug development program. The hERG assay is an indispensable step and a high-quality assay must accompany any investigational new drug (IND) application. While it is the gold standard at present, the hERG assay is too labor-intensive and too low throughput to be used as a screen early in the discovery/development process. A variety of indirect high throughput screens have been used.

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Sudden cardiac death due to non-cardiac drugs is the major safety issue presently facing the pharmaceutical industry and the agencies that regulate it. In recent years, several blockbuster drugs such as terfenadine (Seldane), cisapride (Propulsid), grepafloxacin (Raxar) and terodiline have been withdrawn from major markets, other drugs such as sertindole (Serlect) have been withdrawn prior to marketing and still others such as ziprasidone (Zeldox) have undergone severe labeling restrictions. The issue is transcendental involving at least molecular and systems pharmacology, international regulatory policy and drug development by the pharmaceutical industry.

1. Background

The poster drug for the problem is terfenadine. This drug was the first non-sedating antihistamine and was wildly popular for its non-sedating efficacy in the treatment of allergic rhinitis. Several years after its introduction adverse reports of syncope, polymorphic ventricular tachycardia (torsade de pointes or TdP) and sudden cardiac death began to appear especially with concomitant use of conazole antibiotics. By the mid-1990s, the death toll was mounting and regulatory concern had led to extensive black box labeling restrictions. Ultimately the drug was withdrawn as other, safer, non-sedating antihistamines such as loratadine and

fexofenadine, the active metabolite for which terfenadine is a pro-drug, became available.

TdP is linked to defective repolarization and prolongation of the QT interval of the EKG; at the cellular level the duration of the cardiac action potential (APD) is prolonged. The major membrane currents that might be involved are shown in Fig. 1.

In 1993, Woosley's lab showed that terfenadine blocked the rapidly repolarizing cardiac K^+ current I_{Kr} in feline ventricular myocytes at therapeutically relevant concentrations [1]. During this time, the Sanguinetti/Keating labs showed that the locus for the second most prevalent form of hereditary long QT syndrome (hLQTS) was hERG which expressed a current mimicking the rapidly repolarizing cardiac potassium current I_{Kr} [2]. Subsequently, my lab showed that hERG was the molecular target of terfenadine and we proposed that it might be the target for other non-cardiac drugs with QT liability [3]. At about the same time as terfenadine was raising safety flags, another blockbuster drug, cisapride, used to treat gastro-esophageal reflux (GERD), was linked to QT prolongation, TdP and sudden cardiac death Rampe et al. [4] and Mohammed et al. [5] showed that once again hERG was the molecular target. Hundreds of drugs later it appears that hERG is the only established target for non-cardiac drugs carrying the TdP/prolonged QT liability. Table 1 shows that hERG is the most sensitive to drug block of the potassium channels involved in repolarization. Table 1 also shows an absence of class action effects (cf. terfenadine with fexofenadine and risperidone with sertindole).

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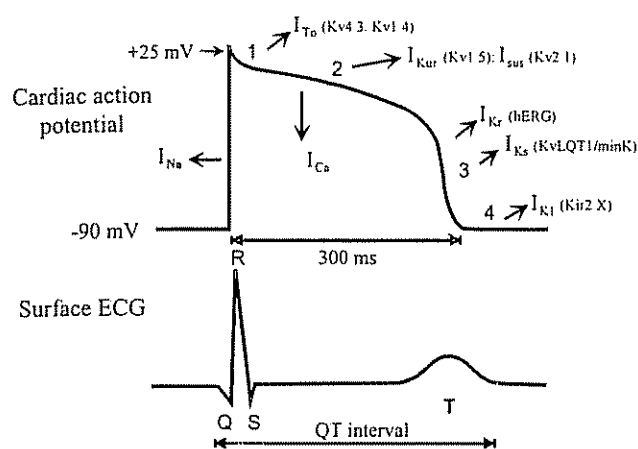


Fig 1 Relationship between cardiac membrane currents, action potential duration and the QT interval of the EKG. The commonly used gene names are in parentheses

Table 1

IC₅₀ values (μM) for non-cardiac drug blockade of cloned cardiac potassium channels [6]

Drug	hERG	Kv1.5	Kv4.3	KvLQT1/minK
Antihistamine				
Terfenadine	0.056	0.367	2.70	4.40
Fexofenadine	13.1	389	112	20.4
Antipsychotic				
Risperidone	0.394	9.50	25.5	9.7
Serindole	0.005	4.00	8.80	0.880
Antibiotic				
Erythromycin	387	ND	606	ND
Prokinetic				
Cisapride	0.044	21.2	9.33	3.39

2. Non-cardiac drugs, sudden cardiac death and the QT conundrum

The adverse event of concern is TdP or monomorphic VT (lumped together as TdP). For the cardiac drug quinidine, the frequency of TdP is about 1:4000 while the frequency of QT prolongation is about 1:20. For non-cardiac drugs, the frequencies are about two orders lower. Because the frequency is so low, TdP was not detected in clinical trials of non-cardiac drugs and the sudden death liability became manifest only during post-marketing surveillance. Faced with this dilemma regulatory bodies have resorted to the use of QT prolongation as a surrogate for TdP. How good is this surrogate? Not very, for the following reasons: first, the QT interval varies with heart rate and there is neither a mechanistic explanation nor an agreed method of correction for this variability. Second, terfenadine and cisapride showed an average QT increase of about 10 ms; this is less than 3% of the regular QT interval and is well within the daily variability of QT in an individual. Third, the relationship between QT prolongation and TdP is a weak power

function and has no threshold to identify imminent danger [7]. Fourth, the mechanism linking QT prolongation to TdP is unknown; prolongation of APD is associated with early after-depolarizations (EADs) due to reactivation of calcium current. EADs may trigger arrhythmia, but TdP may occur absent EADs and maintenance of TdP requires increased transmural dispersion of repolarization and re-entry pathways that have only been speculated upon. Fifth, the relationship may be drug-dependent; for equivalent QT prolongation drugs may differ in the frequency of TdP (sotalol versus dofetilide; amiodarone versus almokalant) [7]. Given all these shortcomings, it is difficult to understand any position that would make QT measurement the *sine qua non* for risk/hazard estimates of drug safety as has been suggested (see <http://www.eudravigilance.org>).

QT prolongation per se reflects defective repolarization involving the currents and channels shown in Fig 1 and it is not surprising that preclinical measurement of drug effects on channel function provides valuable information concerning the potential for QT prolongation liability.

3. Preclinical assays of repolarization liability

As a practical matter, drug developers cannot wait until Phase I clinical trials to determine drug safety. Rather, this determination must be made before an IND submission and preferably earlier in the discovery/lead development program. hERG is the only proven molecular target for non-cardiac drugs that carry a defective repolarization liability, and drug block of the hERG current expressed in heterologous cell lines is a direct test of this propensity. Almost all drugs will block hERG at sufficiently high concentrations so the relevant parameter is the ratio of the IC₅₀ for drug block of hERG to the IC₅₀ or EC₅₀ for the drug at its primary target; the latter translates into the effective therapeutic plasma concentration of the drug which is more appropriate in the denominator. The ratio is referred to as the safety margin or SM. If the ratio is >100, the SM is adequate; if the ratio is <10, the SM is too low, and between 10 and 100, the SM is indeterminate [8]. Judgments must also be made in context. For example, it is possible for a drug such as verapamil to be a potent blocker of hERG, yet

Table 2
Literature reports of IC₅₀s for I_{Kr} blockade [9]

Drug	Lowest I _{Kr} IC ₅₀ (μM)	Highest I _{Kr} IC ₅₀ (μM)	Fold difference
Seldane®/terfenadine	0.056	0.28	2.9
Chlor-Trimeton®/chlorpheniramine	1.1	21	19.1
Allegra®/fexofenadine	21.6	>500	>23.2
Hismanal®/astemizole	0.0009	0.33	367
Claritin®/loratidine	0.173	100	578
Benadryl®/diphenhydramine	0.03	27	900

Table 3
Test substances investigated by ILSI

Test substance	IC ₅₀ value (nmol/l)		
	ChanTest ^a	Zenas ^a	DSTC
Positive			
Bepriidil	24	84	17
Cisapride	21	31	26
Haloperidol	27	93	25
Pimozide	1	35	2
Thioridazine	74	256	43
Terfenadine	9	33	18
Negative			
Amoxicillin	N A	N A	>100000
Aspirin	N A	N A	>100000
Captopril	N A	N A	>100000
Diphenhydramine	1900	5700	997
Propranolol	8200	2800	6658
Verapamil	180	252	199

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^a According to Data Summary Graphs of the Cardiovascular Safety Subcommittee presented at ILSI Workshop in Washington, DC in June 2003 and DSTC data presented at the Society for Pharmacological Safety meeting, 2003

avoid a repolarization liability by also being a potent blocker of inward calcium current. A number of non-cardiac drugs have these mixed ion channel effects. Also, the risk/hazard ratio is clearly different among seasonal hay fever on the one hand and cancer or AIDS on the other. Finally, a me-too drug will have a higher SM barrier to overcome.

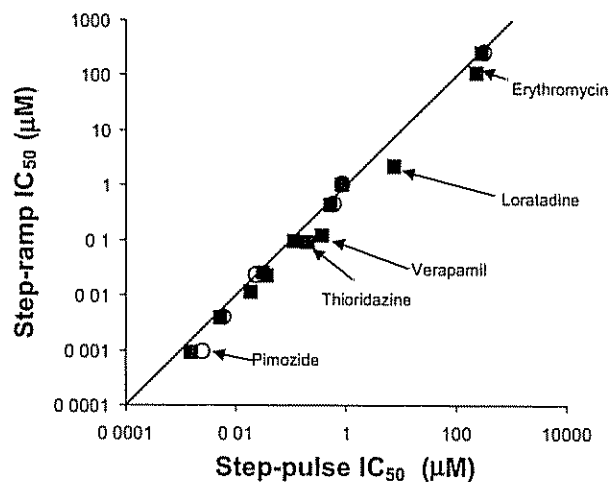


Fig 3 Comparison of IC₅₀s for hERG block between step-ramp and step-pulse protocols. Conditioning step (+20 mV amplitude, 1 s duration) was followed by a repolarizing test ramp (+20 to -80 mV at -0.5 V/s) repeated at 5-s intervals from a holding potential of -80 mV. The step-ramp data were more sensitive.

4. The hERG assay

The *in vitro* assay is straightforward and is done using patch clamp measurements of the hERG current stably expressed in transfected cell lines such as HEK293 and CHO cells. The assay is functional and provides the reference standard by which other methods must be judged. Considerable discrepancies in IC₅₀s have been reported (Table 2).

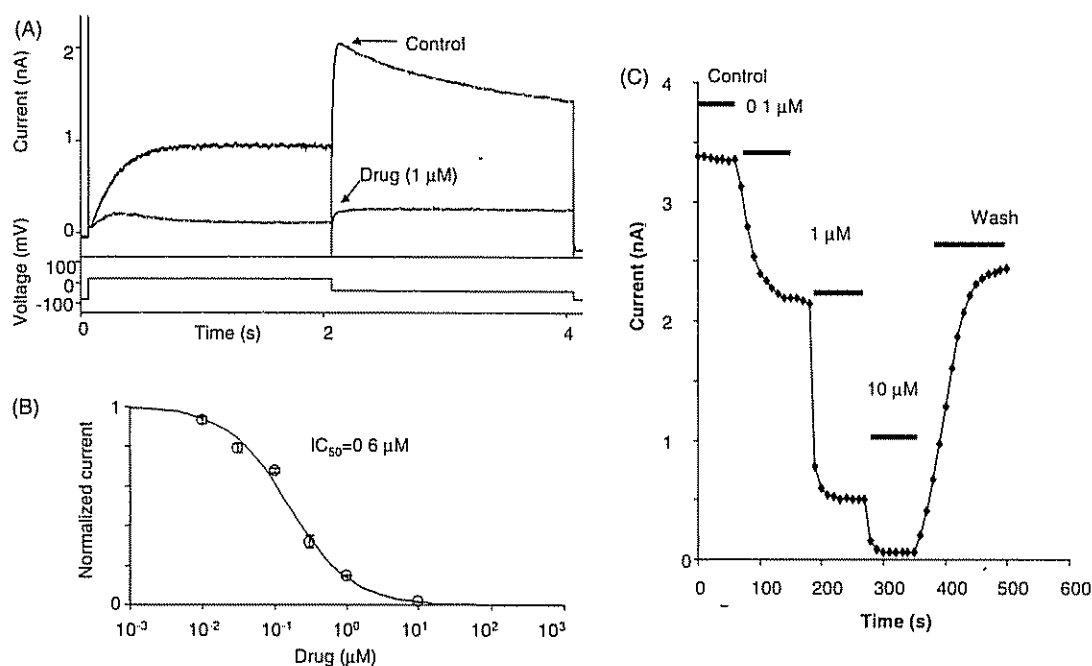


Fig 2 Steady-state block of hERG. (A) hERG currents expressed in stably transfected HEK293 cells using a conditioning prepulse to 20 mV from -80 mV for 2 s and a test potential of -50 mV done at 22 °C at 0.1 Hz. (B) The fit of a simple Hill plot to the fractional block data. (C) The development of steady-state block.

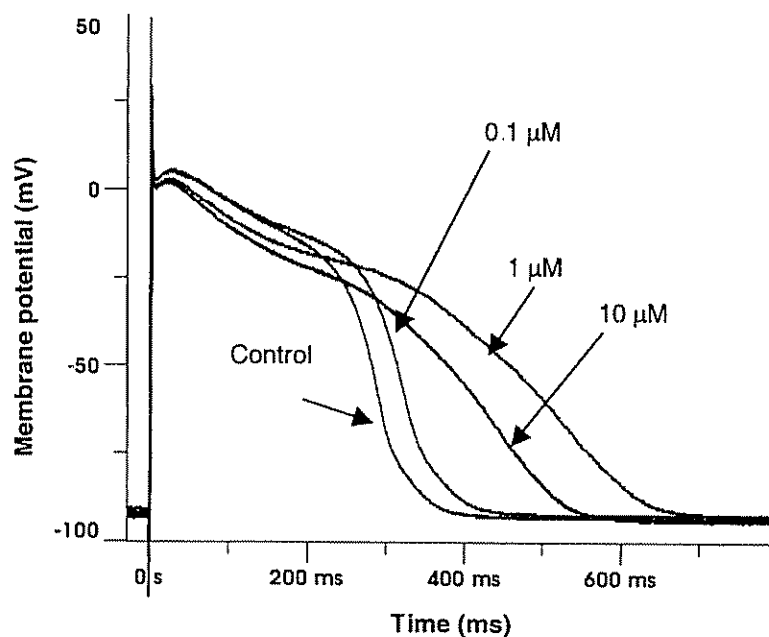


Fig. 4 Biphasic effect of cisapride on dog Purkinje fiber AP. Maximum prolongation of APD occurred at 1 mM. Temperature 36.4 °C, basic cycle length 2 s

When protocols are standardized and block measured at steady-state with minimal rundown (Fig. 2), the differences among labs are minimal (Table 3). Block may be strongly temperature-dependent (e.g., erythromycin and sotalol) and is most conservatively measured using a pulse-ramp method at physiological temperature (Fig. 3).

The hERG assay is labor-intensive and has low throughput. Several methods of automating patch clamp have been devised but the throughput is too low at present to be useful in drug discovery and development. Methods with higher throughput are non-functional and include displacement of high affinity, radioactively-labeled blockers, atomic absorption measurement of rubidium flux and fluorescence detection of voltage-sensitive dyes. Recently, we have introduced a surface expression method called hERG-Lite that takes advantage of the fact that blockers of hERG rescue certain mutations of hERG responsible for hereditary long QT syndrome. This method has the great advantage in that it also detects chemotherapeutic drugs such as arsenic trioxide and geldanamycin [10] that produce hERG/QT liabilities and TdP by inhibition of hERG trafficking.

5. The APD assay

This *in vitro* assay customarily uses APD measurements in Purkinje fibers isolated from the ventricle of a variety of species, the most favored being dog or rabbit. The relative merits of the assay have been described elsewhere [11] but the main shortcoming is access of the drug to the fibers. The rabbit preparation is more sensitive than the dog preparation, probably because access is better. In addition to information on repolarization, the assay measures resting potential and rate of rise of the action potential. Potent block of hERG does

not necessarily establish QT/TdP liability because, as we have noted, blockers such as verapamil may have mixed ion channel effects and block inward calcium and/or sodium currents with equivalent potency thereby offsetting the hERG block and maintaining a normal APD. Other drugs such as cisapride and terfenadine may block hERG with high potency and sodium and/or calcium currents at lower potency, producing a peak followed by a decrease in the relationship between APD prolongation and drug concentration (Fig. 4).

6. Summary

In a minority of cases, hERG block may not cause APD/QT prolongation if it is offset by block of sodium and/or calcium currents. The decision to go forward must be made with this and other considerations that we have described above, kept in mind.

The link between QT prolongation and TdP is tenuous and QT prolongation is a poor surrogate for adverse cardiac events. The full panoply of preclinical and clinical tests are necessary for an informed decision by regulatory authorities.

Acknowledgements

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