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Inhibition of hERG Channel Trafficking: An Under-Explored Mechanism for Drug-Induced QT Prolongation

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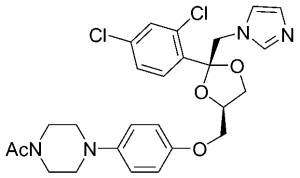
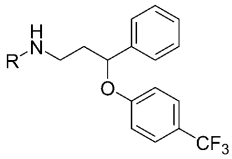
Functional blockage of the cardiac potassium channel encoded by the human *ether-à-go-go-related gene* (hERG or KCNH2) is responsible for the rapidly activating component of the delayed rectifier K^+ current (I_{kr}), which can delay ventricular cell repolarization. As a result of this, the QT interval on an electrocardiogram is prolonged, and can potentially lead to lethal cardiac arrhythmias. This serious cardiotoxicity has resulted in the withdrawal of approved drugs from the market, and discontinuation of clinical trials on development candidates.[1]

Drugs that cause long QT syndrome (LQTS) generally exert a direct blockade of the hERG channel by binding to a common site defined by the key residues Tyr652 and Phe656 in the S6 pore domain of the hERG channel.[2] As measured by the in vitro patch clamp technique, acute inhibition of the I_{kr} current and its recovery are essentially instantaneous, with blockade and relief occurring within minutes. However, a number of drugs, including the commonly prescribed azole antifungal ketoconazole and the antidepressant fluoxetine, a selective serotonin reuptake inhibitor (Figure 1), have recently been shown to induce LQTS by an indirect, chronic inhibitory mechanism that reduces hERG channel expression at the cell surface.[3] It is now apparent that most of the genetic mutations in hERG that cause LQTS do so by this indirect mechanism, denoted as a class 2 effect.[4]

Ketoconazole acutely inhibits the I_{kr} current in hERG-expressing human embryonic kidney (hERG-HEK 293) cells with an IC_{50} value of 1.92 μM. As revealed by Western blot analysis, and by peak tail current measurement, ketoconazole also reduced cell surface expression of the mature, fully glycosylated 155 kD hERG channel after 48 h of incubation, with 50% inhibition observed at concentrations above 10 μM.[5] A greater than 50% reduction in cell surface expression of the hERG channel persisted for up to 48 h after incubation with 30 μM of ketoconazole, and recovery was slow after drug washout, with about 74% of the channels restored after 48 h. The direct and indirect inhibition of the hERG channel by ketoconazole are independent of each other but the effect is additive.



Ketoconazole

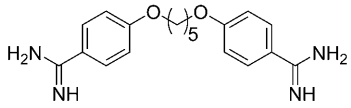


Fluoxetine (R = Me)
Norfluoxetine (R = H)

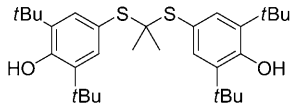
Figure 1. Examples of dual mode inhibitors that directly block hERG channels and also reduce hERG channel expression at the cell surface.

Similarly, the IC_{50} values determined for the acute inhibition of the I_{kr} current in hERG-HEK 293 cells by fluoxetine was 0.7 μM, while that for cell surface expression, as measured by Western blot image density, was 2.7 μM.[6] Norfluoxetine, a metabolite of fluoxetine, was also active in these assays although two to threefold less potent. Following treatment with 30 μM fluoxetine, the reduction of cell surface expression of hERG and recovery took hours, with 100% restoration occurring at ~24 h after drug washout.

Ketoconazole and fluoxetine are thus categorized as dual mode inhibitors of the hERG channel, a property common to many acute inhibitors. Preliminary results using an antibody-based chemiluminescent assay (HERG-Lite®), which measures the cell surface expression of hERG in HEK 293 cells, showed that 20 known direct hERG inhibitors, representing 40% of those tested, also exhibited the indirect mechanism of inhibition that leads to reduced cell surface expression, including: diphenhydramine, terfenadine, tamoxifen, chlorpromazine and lovastatin.[7] However, there are examples of hERG inhibitors that exert their action only by reducing cell surface expression of the protein, most prominently pentamidine, an antiprotozoal agent used for treating *Pneumocystis carinii* pneumonia, and probucol, a cholesterol-lowering agent (Figure 2). Significantly, pentamidine[8] and probucol[9] have also been shown to reduce I_{kr} tail current, and prolong the action potential duration in guinea pigs and neonatal rat ventricular myocytes via this indirect mechanism,



Pentamidine



Probucol

Figure 2. Examples of drugs that cause LQTS via reduced hERG channel expression at the cell surface.

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with potencies $<30\ \mu M$. The Na^+/K^+-ATPase-inhibiting cardiac glycosides (e.g. digoxin and digitoxin),[10] that are used for treating atrial fibrillation and congestive heart failure, and the anti-inflammatory and, antioxidant triterpene celastrol,[11] also demonstrate inhibition of hERG cell surface expression, however with nM potencies.

QT prolongation caused by fluoxetine is only occasionally observed in the clinic, often the result of drug overdose, and the incidence rate of ketoconazole induced LQTS is also low. As suggested by the in vitro IC_{50} values, the maximum unbound concentration of both drugs in plasma after therapeutic doses (<50 nM) is not sufficient to produce either the acute or chronic adverse cardiac effect.[5,6] In contrast, pentamidine and probucol have been known since the 1980s to frequently cause LQTS clinically through an unknown mechanism. The mean free drug plasma concentrations of pentamidine ($>0.5\ \mu M$) and probucol (35–75 μM) occurring after long-term treatment are sufficiently high to cause the chronic effect on cell surface channel expression.[8a,9] The in vitro incubation results are also consistent with the clinically observed delayed onset of LQTS after treatment with ketoconazole[12] and pentamidine[13] of 3 and >12 days, respectively, and recovery after withdrawal of 4 and >7 days, respectively.

The precise mechanism by which these drugs decrease the cell surface expression of the hERG channel protein remains to be determined.[3] However, inhibition of protein synthesis by these drugs can be ruled out since the density of the 135 kD nascent hERG channel protein synthesized in the endoplasmic reticulum was not affected. Studies with ketoconazole[5] and fluoxetine[6] using Tyr652/Phe656 mutant channels indicated that binding in the Tyr652/Phe656 site is also not involved in this type of inhibition. The literature reports to date show that the indirect inhibitors of hERG are structurally diverse and a toxicophore is not yet obvious.

These and other reports indicate that inhibition of hERG channel trafficking is an alternative mechanism for drug-induced LQTS. However, current compound profiling for hERG inhibition during the drug discovery process rarely encompasses this mechanistic possibility. Implementation of screening for this type of cardiotoxicity should therefore be considered. Given the delayed onset of LQTS induced by this novel mechanism, it is possible that inhibitors of hERG channel cell surface expression may only be identified by long-term in vivo safety studies.

Keywords: drug safety · hERG channels · cell surface expression · long QT syndrome · ion channels

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