Cellular models for fundamental and applied biomedical research

Liu, J.

Chapter 6

Appendix 1

hERG channel (K_v11.1) modulators that enhance dissociation of drugs from their blocking receptor

Saman Rezazadeh, Henry Duff

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Sudden cardiac death secondary to drug-induced long QT syndrome is a major safety concern and has led to withdrawal of several high-profile drugs, such as cisapride and astemizole, from the market. Although these drugs have different chemical structures, they all block the rapid delayed rectifier K^+ current (I_{Kr}) with high potency. The hERG channel (Kv11.1) encoded by the *hERG* gene is responsible for this current. This channel has a high affinity for wide spectrum of compounds compared with other ion channels mainly because of the presence of unique aromatic amino acids in the S6 domain of hERG.[1] This in turn has created a major challenge for development of new drugs.[2] In fact, it is now a routine practice in the pharmaceutical industry to screen compounds for their ability to block hERG early in preclinical safety assessments according to the Food and Drug Administration guidelines. This in turn leads to elimination of a large number of potentially beneficial compounds from chemical libraries.

Interestingly, screening libraries of compounds for their blocking effect on hERG has resulted in discovery of many hERG activators/modulators. These drugs increase current flow through hERG via various mechanisms that include slowing of deactivation, removal of inactivation, and facilitation of activation.[3] In theory, hERG channel activators could have the potential to normalize the QT interval in acquired or congenital long QT syndromes.[4] In fact, the proarrhythmic effects of dofetilide can be reversed, in vitro, by application of hERG channel activators.[5] The draw back in the use of hERG activators clinically has been their inherent ability to shorten the QT interval, possibly to the point of causing arrhythmia.

Given the strain that is placed on drug discovery and also withdrawal of several previously successful drugs because of their QT prolonging properties, it is important to develop reproducible, physiological, and clinically translatable techniques to examine the effect of hERG channel activators and their interaction with long QT inducing drugs. In this issue, Yu *et al*,[6] by using membranes of HEK 293 cells expressing hERG for in vitro radioligand assay, show that a newly synthesized hERG modulator (LUF7244) diminishes the binding affinity of potent hERG blockers, such as dofetilide, astemizole, sertindole, and cisapride. Increased concentrations of this modulator resulted in reduced specific binding of radioactive dofetilide, with Hill coefficient >1, suggesting allosteric interaction rather than direct competition for the same binding site. This notion was fortified by demonstration of altered dissociation rate constant in the presence of LUF7244. This is in agreement with previous findings that openers/modulators may exert their effect by interacting with the channel at sites separate from the pore, where the class III drugs bind.[7] Interestingly, the authors showcase a clever mean to assay the effect of LUF7244 on hERG channel blockers in a physiological setting by using cultured neonatal rate ventricular myocytes. They used high-resolution optical mapping to record action potentials from cells grown to confluence. With this technique, LUF7244 reversed the prolonging effect of astemizole on action potential duration and prevented induction of early after depolarization. Surprisingly, in the absence of hERG blockers, LUF7244 did not shorten action potential duration.

Lack of shortening of action potential duration by LUF7244 was not examined further in their article. This could be because of off-target effect of LUF7244 on other ion channels. For example, NS1643, a known activator of hERG, blocks KCNQ1

current, which is responsible for slow delayed rectifier K^+ current (I_{Ks}).[8] If the ratio of blocking activity at hERG and KCNQ1 balance each other out, then action potential duration will not change significantly, which could be a beneficial feature as it would prevent short QT syndrome. Therefore, it is important to assess the effect of LUF7244 and future hERG activators on a range of ion channels to ensure the absence of unwanted off-target effect.

This article raises 2 interesting questions. First, can hERG modulators/activators be used in combination with drugs that are known to block hERG to mitigate the long QT side effect? This has an immense clinical prospect because it will allow for reintroduction of previously successful drugs and can potentially accelerate drug discovery and development. A significant number of chemicals are eliminated from libraries during drug discovery because of their affinity to hERG. Therefore, by having the ability to counteract this effect, there will be more compounds available to screen for use in variety of diseases. In the future, through mapping the binding site and mechanism of action of various activators, more potent activators will surely be developed.

The second question is can the assays developed by Yu *et al*[6] be used as a high throughput screening strategy? Although patch clamp remains the gold standard in studying the biophysical properties of channels and determining the details by which drugs interact with them, it is technically challenging with low throughput. Here, the authors demonstrate the use of cultured neonatal rat myocytes and optical mapping to assess for action potential duration. The advantage of this technique over existing assays is that it is a physiological screen, capable of determining the effect of a compound on the action potential duration and potential for evoking early after depolarization. Even so, patch-clamp studies of LUF7244 seem essential to clarify its detailed molecular mechanism(s) of action. A detailed understanding of its state-dependent pharmacological affects seems critical. Sitedirected mutagenesis may provide insights as to the topology of its allosteric-binding site on the hERG (KCNH2) channel. Enhanced description of its electrophysiological effects seems an important next step.

A recent article by Yu *et al*[9] proposes interesting potential mechanism(s) for increased dissociation of 3H-dofetilide and 3H-astemizole from the hERG channel by an allosteric modulator (LUF6200). Potassium ions both enhance the allosteric effect of LUF6200 and shift the LUF6200 dose–response curve to the left. Furthermore, their studies suggested a positive cooperativity between LUF6200 and potassium ions. Residence of potassium ions in the selectivity filter of hERG is critical to the C-type inactivation process. Moreover, C-type inactivation state seems to play a pivotal role in stabilizing the dofetilide-associated state of the channel by preventing its dissociation. Alteration of the residence of potassium ions near the C-type inactivation gate (selectivity filter) would shift voltage dependence of inactivation. Modifying C-type inactivation would thus allow dissociation of dofetilide and astemizole from its binding site in the pore. Patch-clamp studies will be necessary to test this hypothesis. Even so, there could be >1 binding site in hERG or binding sites in other ion channels that can indirectly alter dissociation of dofetilide and astemizole from their binding sites. Further studies of this interesting new compound are warranted.

Disclosures

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