Stem Cell–Derived Cardiomyocytes as a Tool for Studying Proarrhythmia

A Better Canary in the Coal Mine?

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Proarrhythmia is a leading cause of hospitalization for adverse drug events, and screening for proarrhythmic potential resulting from QT interval prolongation has become a major component of the development process for any new drug candidate. Conventional wisdom, now embedded in regulatory guidelines from the Food and Drug Administration and other regulatory agencies, holds that drug block of the rapid component of the cardiac delayed-rectifier potassium current, $I_{Kr}$, is the major proximate mechanism underlying prolongation of cardiac action potentials, an effect that leads to early afterdepolarizations in vitro and is manifest on the surface ECG as QT prolongation and occasionally torsades de pointes.\textsuperscript{1,2} As a result, regulatory guidance suggests an early look at $I_{Kr}$ blocking potency of new drug entities, even recognizing that other mechanisms, some newly described,\textsuperscript{3,4} may contribute.

However, the correlation between $I_{Kr}$ block and QT interval prolongation is imperfect. Multiple mechanisms may underlie this “disconnect,” and a reasonable generalization is that $I_{Kr}$ is but one (albeit important) component of normal cardiac repolarization, and abnormalities in expression or function of other components of repolarization may, in turn, modulate the net effect of $I_{Kr}$ block on action potentials in cardiac myocytes and on QT interval and arrhythmia susceptibility in the whole heart. The logical extension of this line of reasoning is that screening for proarrhythmic potential should include the evaluation of not only drug effects on individual ionic currents such as $I_{Kr}$ contributing to repolarization but also the integrated effect of drugs on action potentials in vitro and on the QT interval in animal models and in humans. In conventional drug development programs, this is commonly accomplished by comparing the effects of a candidate drug molecule on QT intervals in human subjects with those of placebo and a positive control in a so-called thorough QT study.

The question of identifying individuals at unusually increased risk was approached by studying the effects of the potent $I_{Kr}$ blocker cisapride on action potentials in these cardiomyocytes. Before drug exposure, action potentials in long-QT syndrome cells were longer than those in other groups, HCM cardiomyocytes displayed delayed afterdepolarizations suggestive of abnormal intracellular calcium control, and long-QT syndrome cardiomyocytes displayed both early and delayed afterdepolarizations.

The development of technologies to generate human cardiomyocytes from embryonic stem cells and induced pluripotent stem cells (iPSCs) raises 2 tantalizing prospects for work in drug-induced proarrhythmia: that proarrhythmic drugs might be readily identified early in a development program and that individuals at high risk for proarrhythmia could be identified before drug exposures. The Wu laboratory at Stanford\textsuperscript{9} reports in this issue of Circulation interesting steps to enabling this twin vision, complementing recent reports from others using this approach.\textsuperscript{10,11} iPSC-derived cardiac ventricular myocytes from patients with long-QT syndrome caused by a KCNQ1 mutation, hypertrophic cardiomyopathy (HCM) resulting from a β-myosin heavy chain (MYH7) mutation, or dilated cardiomyopathy caused by a troponin T (TNNT2) mutation were compared with iPSC-derived cardiomyocytes from family members without mutations and with embryonic stem cell–derived cardiomyocytes. Before drug exposure, action potentials in long-QT syndrome cells were longer than those in other groups, HCM cardiomyocytes displayed delayed afterdepolarizations suggestive of abnormal intracellular calcium control, and long-QT syndrome cardiomyocytes displayed both early and delayed afterdepolarizations.

Using Stem Cell–Derived Cardiomyocytes to Study Proarrhythmia

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The question of identifying individuals at unusually increased risk was approached by studying the effects of the potent $I_{Kr}$ blocker cisapride on action potentials in these models. The drug produced early afterdepolarizations at much lower concentrations in long-QT syndrome and in HCM cardiomyocytes than in normal or in dilated cardiomyopathy...
cardiomyocytes. This experiment thus not only complements previous work highlighting the protective role of a robust $I_{Kr}$ but also supports the clinical impression that patients with HCM may be at increased risk for drug-induced torsades. Moreover, the result is in agreement with reports from other laboratories, also using iPSC-derived cardiomyocytes in drug screening, that highlight the role of altered repolarization reserve in mediating proarrhythmic risk.10

The utility of these cardiomyocytes in screening for proarrhythmic drugs was further assessed by examining the effects of verapamil and of alfuzosin on action potentials from normal individuals. Both drugs have $I_{Kr}/QT$ “signals” of sorts, but neither is recognized as likely to produce torsades. Verapamil blocks both $I_{Kr}$ and L-type calcium channels and was associated here with minimal (and statistically insignificant) action potential prolongation.12 This finding is in keeping with previous studies showing that verapamil can in fact reverse early afterdepolarizations in vitro presumably because of the primary role of its calcium channel blocking actions.13 Alfuzosin is an α-blocker developed for urologic indications found to increase heart rate and to prolong QT marginally in a thorough QT study. The mechanism for this signal has been somewhat controversial, especially because the drug is not a known $I_{Kr}$ blocker. One possibility is that alfuzosin increases a late component of the sodium current to slightly prolong QT; another is that there is little true effect on repolarization, and the thorough QT signal is largely an artifact of inaccurate rate correction.14 In the stem cell–derived cardiomyocytes, alfuzosin prolonged action potentials to an extent very similar to that of verapamil, although in this case the signal was statistically significant.

A fourth drug, the $I_{KATP}$ channel opener nicorandil, was antiarrhythmic in the long-QT syndrome myocytes but regenerated afterdepolarizations at high concentrations. Interestingly, the drug has been reported to reverse long-QT–related arrhythmias in experimental models15 and to be associated with ventricular fibrillation at high doses in humans. Thus, the drug studies generally parallel clinical reports of drug action, and the nicorandil result highlights the notion that drugs may be proarrhythmic through non–long-QT–related mechanisms.16

### Unanswered Questions

The clinical phenotypes seen in the MYH7 and TNNT2 patients from whom these cardiomyocytes were derived have briefly been described previously.17,18 One interesting finding is that penetrance varies among mutation carriers, and although not directly germane to the question of drug sensitivity, this finding highlights the potential utility of studying cardiomyocytes from individual subjects to dissect underlying mechanisms. Notably, TNNT2 mutations, modeled here as a cause for dilated cardiomyopathy, also are associated with HCM.

iPSC-derived cardiomyocytes display atrial, nodal, or ventricular-like action potential configurations; only those with ventricular-like configurations were studied here. Nevertheless, the action potential characteristics are atypical for the adult mature cardiomyocyte; the cells display spontaneous phase 4 depolarization, which is not seen in normal ventricular myocytes, and seem to display relatively depolarized resting potentials, although these data are not reported. The authors also report ion channel profiling in the normal and disease model myocytes, and these data further support the contention that the myocytes are relatively immature. Specifically, there is much reduced expression of KCND3 (responsible for the transient outward current), CACNA1C (L-type calcium channel), and KCNJ2 (a major contributor to the inward rectifier and thus a determinant of resting potential). Accordingly, the cells studied here cannot be designated normal human adult ventricular myocytes, and the extent to which data obtained in this model can be extrapolated to the human condition therefore is not established.

### Next Steps

A number of findings here immediately generate questions for further study. For example, an interesting result is the apparent overexpression of KCNJ3, KCNJ5, and KCNA5 in HCM myocytes. Expression of these genes is thought to underlie the expression of atrium-specific ion currents ($I_{Ks,ACH}$ and $I_{Kr}$), and it is provocative that the atrial natriuretic factor gene is also overexpressed in HCM and in the HCM-derived cell lines. The mechanisms and electrophysiological consequences of these findings deserve further exploration.

Similarly, the method may lend itself to understanding why different TNNT2 mutations cause HCM or dilated cardiomyopathy. The observations of very frequent delayed afterdepolarizations in the β-myosin heavy chain myocytes (not seen in the troponin T myocytes) support the idea that mutant sarcomeric proteins in HCM contribute to an arrhythmogenic substrate by perturbing intracellular calcium control.19 The development of delayed afterdepolarizations by a high concentration of nicorandil may be a window into non–long-QT–related proarrhythmic drug actions.

Verapamil and alfuzosin, drugs not associated with much QT interval prolongation or arrhythmias, did not produce much action potential prolongation or afterdepolarizations in the cells. This finding is reassuring for the use of iPSC-derived myocytes as a potential screening tool for new drugs or for individual patients, as is the demonstration of reduced repolarization reserve and marked proarrhythmic effects by cisapride, a positive control in this experiment. However, the utility of these cell lines in screening for proarrhythmic potential of new drug entities remains far from established. The absence of a Purkinje myocyte and the question of whether this system will allow the assessment of variable susceptibility to proarrhythmic effects in the absence of defined and well-characterized genetic lesions remains unanswered. Heart failure is a major contributor to enhanced susceptibility to proarrhythmia, and the extent to which HCM and dilated cardiomyopathy models mimic such acquired disease remains to be determined. Studies in single cells do not recapitulate the multicellular and highly heterogeneous substrate that is thought to predispose to many clinically important arrhythmias.
Good Experiments Always Raise More Questions

All good experiments are designed to answer specific questions. If the question is, “Does this drug block \( I_{Kr} \)?” then the best system with which to answer that question is one in which human \( I_{Kr} \) is faithfully recapitulated in splendid isolation. This is a great advantage of the heterologous expression systems. If the question is, “Does this drug increase the likelihood of long QT-related proarrhythmia?” then studies of the effect of the drug on action potentials, generated by a highly interactive ballet of multiple ion currents and other electrogenic phenomenon, are more appropriate. The closer we come to the human situation, the more likely it is that we can confidently answer the question for patients. The cardiomyocytes studied here are an important step in that direction, but work remains to be done to develop robust systems to develop cells with the fully adult phenotype and to extend the range of drugs and of cardiomyocyte lines evaluated. Indeed, early studies from others highlight the failure of these “first-generation” iPSC-derived cardiomyocyte lines evaluated. Indeed, early studies from others highlight the failure of these “first-generation” iPSC-derived cardiomyocytes to faithfully reproduce proarrhythmic potential when more drugs with differing mechanisms were derived.10 Meanwhile, these data show the path forward and, as with all good experiments, generate findings that are unexpected and therefore provide truly interesting fodder for further experimentation.

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Disclosures

None.

References

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