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Review Article

Complementarity between Arrhythmia Mechanisms Found *in Silico* and in Genetic Models of N588K-hERG Linked Short QT Syndrome

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ABSTRACT

Congenital Short QT Syndrome (SQTS) is a rare but dangerous condition involving abbreviated ventricular repolarization and an increased risk of atrial and ventricular arrhythmias. Taking the example of the first identified SQTS mutation, N588K-hERG, we consider briefly the basic science approaches used to obtain an understanding of the mechanism(s) of arrhythmogenesis in this form of the syndrome. A combination of recombinant channel electrophysiology and *in silico* simulations has provided insights into causality between the identified mutation, accelerated repolarization and increased susceptibility to re-entry in N588K-hERG-linked SQTS. Subsequent studies employing a transgenic rabbit model or human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) have further demonstrated mechanisms predisposing to re-entry, spiral wave activity and arrhythmia in intact tissue. The complementarity between the findings made using these different approaches gives confidence that, collectively, they have identified major arrhythmia mechanisms and their potential mitigation by Class I antiarrhythmic drugs in this form of SQTS.

Introduction

Congenital short QT syndrome (SQTS) is a rare but dangerous condition characterized by abbreviated ventricular repolarization; it is associated with an increased risk of atrial and ventricular arrhythmias and of sudden death [1, 2]. Whilst a number of different genes have been associated with the SQTS, the most commonly found mutations in SQTS patients to date are to *hERG* (*human-Ether-à-go-go Related Gene*; alternative nomenclature *KCNH2*) encoded potassium channels [2-4]. hERG channels mediate the rapid delayed rectifier potassium (K^+) current (I_{Kr}), which works in conjunction with other ion channels to repolarize the ventricular action potential [5-7]. Mutations to hERG have long been established to be responsible for the LQT2 form of congenital long QT syndrome (LQTS), whilst a unique propensity of hERG channels to pharmacological blockade underpins the channel's involvement in the drug-induced form of LQTS [8, 9].

The first SQTS variant (SQT1) to be identified was found in two families in which different missense mutations led to a common amino acid substitution (Asparagine → Lysine; N588K) in the external S5-Pore region of the channel [10]. This region is important to rapid voltage-dependent inactivation of the hERG channel and experiments on heterologously expressed recombinant hERG channels demonstrated conclusively that the N588K mutation produces a marked positive shift in voltage-dependent inactivation of hERG current (I_{hERG}) that results in a marked increase in outward potassium current; this in turn accelerates repolarization and abbreviates refractoriness [10-13]. The attenuated voltage-dependent inactivation in N588K hERG channels makes them less amenable to pharmacological blockade by Class III selective I_{Kr} inhibiting antiarrhythmic drugs (a drug class that includes sotalol and dofetilide), though the channels retain some sensitivity to the Class Ia antiarrhythmics quinidine and disopyramide [10, 14-16]. Consistent with this, SQT1 patients with the N588K mutation are unresponsive to sotalol but have been reported to respond favourably to quinidine and disopyramide [3, 10, 15, 17, 18].

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The understanding of underlying arrhythmia mechanisms in the SQTS has relied for a long time on pharmacological models of the SQTS or *in silico* studies using cell and tissue electrophysiological models. The N588K SQT1 mutation is the best studied SQTS mutation in this regard [2, 19]. Unfortunately, arrhythmia mechanisms in hERG-linked channelopathies cannot reliably be studied using genetically modified mice, as mice have evolved with high heart rates, abbreviated ventricular action potentials (APs) and ventricular repolarisation mechanisms that differ from those in humans, without a reliance on I_{Kr} [20]. The use of single cell ventricular AP models has demonstrated a causal link between the N588K mutation and AP shortening, whilst tissue models incorporating transmural heterogeneity in I_{Kr} were able to reconstitute QT interval shortening and increases in T wave amplitude similar to those seen in patients [13, 21].

Maximal transmural heterogeneity was found to be augmented at some locations in ventricular tissue models and refractoriness was abbreviated as a consequence of AP shortening [13]. In 2D and 3D models, this led to a reduced substrate size required to support re-entry and increased spiral/scroll wave lifespan [13]. The subsequent incorporation of electromechanical coupling led to reductions in Ca^{2+} transients and contractile force under conditions simulating the N588K mutation and altered timing of maximal mechanical deformation in 3D tissue models [22]. Consistent with this, a modest reduction in ventricular myocyte shortening has been observed to accompany AP shortening induced by a pharmacological hERG activator [2]. Such findings are qualitatively consistent with modest systolic changes observed using Doppler imaging and speckle-tracking echocardiography in SQTS patients [23]. A simulation approach has also been used to explore the atrial arrhythmia substrate and its susceptibility to modification by Class I antiarrhythmic drugs in N588K-linked SQTS [24].

Recently, an animal model of N588K-linked SQTS has been published using a species with repolarization mechanisms more similar to those in humans: transgenic rabbits were generated that expressed N588K hERG channels [25]. This model showed abbreviation of the QT interval, AP duration, atrial and ventricular refractory periods and a decrease in slope of the QT/RR relationship [25]. These changes are consistent with the clinical phenotype in the syndrome, though tall T waves seen in humans were not evident in the rabbit ECGs [25]. In contrast with *in silico* and patient data systolic function was not altered in SQT1 rabbits, though diastolic relaxation was enhanced [22, 23, 25]. Inducibility of ventricular tachycardia (VT)/ ventricular fibrillation (VF) was increased in the SQT1 model, whilst quinidine prolonged AP and QT interval duration [25]. One intriguing finding was the presence of some ionic current remodelling in myocytes from SQT1 rabbits: the inwardly rectifying potassium current, I_{K1} was decreased (which would partially oppose the gain-of-function effect of increased I_{Kr} in SQT1), whilst the slow delayed rectifier K^+ current (I_{Ks}) showed a small increase (which could be synergistic with the increased I_{Kr} in SQT1) [25]. Such observations could only be made using an experimental and not *in silico* approach, though the relevance of this finding to SQT1 in patients is not yet clear.

Another promising experimental approach to the study of arrhythmia mechanisms in the SQTS has been the generation of mutation-specific human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes. El-Battrawy *et al.* have generated hiPSCs from SQT1 (N588K) patient and control fibroblasts and derived cardiomyocytes (hiPSC-CMs) from these [26]. Consistent with the simulation and transgenic rabbit findings discussed above, increased I_{Kr} density and shortened AP duration were seen in N588K-hiPSC-CMs compared to control hiPSC-CMs. Abnormal Ca^{2+} transients and arrhythmic activities were also evident and quinidine prolonged AP duration [26]. Carbachol was found to exacerbate arrhythmic events in N588K-hiPSC-CMs and quinidine mitigated this effect. There was no change in mRNA for *KCNJ2* or *KCNQ1*, which respectively encode I_{K1} and I_{Ks} channel subunits, but mRNA for hERG itself and *CACNA1C* (L-type Ca channel) and *KCND3+KCHIP2* (transient outward potassium channel) were all increased in N588K-hiPSC-CMs [26]. A limitation in the use of hiPSC-CM is their well-known immature phenotype, evidenced here by the fact that cells were spontaneously active, which is not the case for mature ventricular myocytes [26].

Nevertheless, the overall phenotype seen is broadly consistent with those from other approaches. Subsequent use of N588K-hiPSC-CMs has probed the potential for additional pharmacological approaches to this form of SQT1, finding potential utility for ivabradine, ajmaline and mexiletine, at least *in vitro* [27]. Of course, the hiPSC-CM approach has wider applicability than solely to the N588K hERG mutation and it has also been used to confirm a causal relationship between the T618I hERG gain-of-function SQT1 mutation and abbreviation of repolarization [28, 29].

A further elaboration of the hiPSC-CM approach to studying SQT1 has come from the combination of experiments on individual hiPSC-CMs with work on 2 dimensional hiPSC-CM sheets [30]. Electrophysiological characteristics have been compared at the cell and multicellular levels in an hiPSC-CM line from a patient with N588K-hERG-linked SQT1 and isogenic control and healthy hiPSC-CM preparations. As may be anticipated, increased I_{Kr} together with abbreviated repolarization and refractory periods were observed in hiPSC-CM expressing the N588K mutation [30]. Optical mapping of SQT1 hiPSC-CM sheets revealed abbreviated action potential duration (APD) and excitation wavelength, impaired APD rate-adaptation and increased inducibility of sustained spiral waves [30]. Spiral waves had accelerated rotation and increased stability, frequency, stationarity and wave front curvature near their cores. Quinidine and disopyramide, but not sotalol, prolonged APD and suppressed arrhythmia [30].

There is striking concordance between the results of this study and earlier published work using *in silico* electrophysiological simulations of N588K-hERG-linked SQT1 [13, 30, 31]. In simulations, modification of I_{Kr} based on N588K hERG channel data led to abbreviated APD and effective refractory period (ERP), flattening of APD and ERP restitution (i.e. impaired APD rate-adaptation) [13]. In 2D and 3D tissue simulations, SQT1 reduced the excitation wavelength but increased tissue susceptibility to re-entry with higher dominant frequencies [13]. When known actions of quinidine and disopyramide were incorporated into SQT1 simulations, both drugs prolonged QT interval (due to I_{Kr} block), ERP (due to a combination of I_{Kr} and Na current (I_{Na}) block), re-entry wavelength and exhibited antifibrillatory effects [31].

The consideration of what experimental and *in silico* approaches have collectively revealed about N588K-hERG-linked SQT1 is instructive in several respects. First, the mechanistic insights into arrhythmogenesis in SQT1 from transgenic rabbit and hiPSC-CM preparations are largely in agreement with those obtained from earlier computer simulation work. This engenders confidence in the conclusions reached from the

incorporation of data from experiments on recombinant channels into biophysically accurate computer simulations using human cardiac electrophysiology models. Perhaps the only significant potential deficit in this approach is that on its own it cannot detect potential accompanying electrical remodelling that may be observed using hiPSC-CMs or transgenic animal approaches [25, 26]. That said, it is notable that the remodelling seen in N588K hERG transgenic rabbits differed from that in hiPSC CMs expressing the same SQT1 mutation and the arrhythmia mechanisms suggested from hiPSC-CM sheets are similar to those seen in computer models of the same mutation that did not incorporate remodelling of other channels [13, 25, 26, 30].

Of the different approaches used, recombinant channel electrophysiology together with computational modelling is likely to be the least expensive, whilst generation and study of mutation-specific transgenic rabbits likely to be the most expensive, although of potentially high value for considering responses at the whole heart level. The small size of native I_{Kr} in both hiPSC-CMs and adult cardiac myocytes, together with the presence of overlapping conductances, is likely to make detailed interrogation of changes to channel kinetics due to gain of function mutations much more difficult than is the case for experiments using recombinant channels expressed in appropriate mammalian cell lines. This limitation to native cardiac myocytes use may be exacerbated for mutations that impair I_{Kr} inactivation, as this can impair selective I_{Kr} blocker action and thus measurement of I_{Kr} as a drug-sensitive current could, at least in principle, underestimate native magnitude under gain-of-function mutant conditions.

Conclusion

It is reasonable to conclude that an initial approach to investigating pathogenicity of gain-of-function hERG mutations, as considered here for the N588K-hERG-linked SQT1 mutation, is to quantify effects on recombinant channel function, then to use those data to interrogate causality in human cardiac AP models. The findings of such studies can then subsequently be further validated/interrogated using mutation-specific hiPSC-CM lines, potentially at both the cellular and multicellular levels. The feasibility of the future *routine* use of genetically modified rabbits (or indeed another appropriate model species) for this kind of work is likely to depend on technological advances to increase cost- and time-effectiveness of the approach. Ultimately, the ability to combine multiple complementary approaches is likely to highlight common mechanistic findings that shed light onto arrhythmogenic mechanisms and approaches to their possible treatment in such syndromes.

Conflicts of Interest

None.

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