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GENE THERAPIES FOR ARRHYTHMIAS IN HEART FAILURE

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Abstract

In this article, we review recent advances in our understanding of arrhythmia mechanisms in the failing heart. We focus on changes in repolarization, conduction, and intracellular calcium cycling because of their importance to the vast majority of clinical arrhythmias in heart failure. We highlight recent efforts to combat arrhythmias using gene-based approaches that target ion channel, gap junction, and calcium cycling proteins. We further discuss the advantages and limitations associated with individual approaches.

Keywords

Heart Failure; Arrhythmias; Conduction; Repolarization; Gap Junctions; SERCA2a; Gene Therapy

Heart failure (HF) is a major public health epidemic worldwide [60]. Malignant arrhythmias account for a significant portion of HF-related deaths [10, 64]. Our incomplete understanding of fundamental arrhythmia mechanisms in the failing heart has hampered the development of effective pharmacological treatments [23]. In fact, the pro-arrhythmic tendency of some pharmacological agents has resulted in the premature termination of the Cardiac Arrhythmia Suppression Trial (CAST), after a substantial increase in arrhythmic deaths was noted in post myocardial infarction (MI) patients treated with encainide or flecainide compared to placebo [22]. These pro-arrhythmic effects may arise from the heterogeneity of ion channel expression and function across regions and layers of the heart [4, 61], the nonspecific nature of most pharmacological agents used to target ion channels, the complex cross-talk between individual ion channels, and the dynamic nature of remodeling that occurs in the context of left ventricular (LV) dysfunction [33]. In this article, we review advances in our understanding of arrhythmia mechanisms in the failing heart and illustrate how improved knowledge of the molecular basis of these arrhythmias can be translated into novel gene-based therapeutic approaches.

CLASSIFICATION OF ARRHYTHMIA MECHANISMS

Arrhythmia mechanisms are classified into 3 broad categories, namely enhanced automaticity, triggered activity, and reentry [68]. The first two are cellular phenomena whereas the latter is a property of the cardiac network. Enhanced automaticity refers to

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acceleration in the spontaneous firing rate of myocytes whether in the sinoatrial node or elsewhere. When the automaticity of ventricular myocytes, which are normally quiescent, is elevated in response to disease, irregular activation patterns can arise. Indeed, abnormal wavefronts originating from ventricular loci can compete with or collide against wavefronts emanating from the sinoatrial node. On the other hand, triggered activity refers to calcium mediated premature action potentials that arise from so-called early or delayed afterdepolarizations. These extra beats register as premature ventricular contractions or short runs of ventricular tachycardia (VT) on the surface electrocardiogram (ECG). The third and most common mechanism (i.e. reentry) is a multi-cellular process that involves one or more excitation wavefronts that circulate around zones of refractory tissue or rotate as spiral waves. All three mechanisms are operative in the failing heart, heightening the susceptibility of patients to sudden cardiac death. While triggered activity and reentry are fully independent mechanisms they often occur in combination. Importantly, complex arrhythmias such as those observed in most etiologies of HF are dependent on the presence of: 1) an arrhythmogenic trigger, and 2) an appropriate electrophysiological substrate that readily converts an “innocent” premature beat to a lethal, sustained arrhythmia.

In what follows, we provide an overview of key electrophysiological abnormalities in the failing heart. We focus on changes in repolarization, conduction, and intracellular calcium cycling because of their importance to the vast majority of arrhythmias in HF [34, 45]. Where appropriate, we highlight recent efforts to combat arrhythmias using gene-based approaches that target ion channel, gap junction, and calcium cycling proteins. We further discuss the limitations associated with individual approaches.

ALTERED REPOLARIZATION IN HEART FAILURE

An electrophysiological hallmark of the end-stage failing heart is a prolonged action potential duration (APD), reflecting delayed terminal repolarization of the cardiac myocyte [64, 65]. This fundamental change in myocyte biology underlies QT-interval prolongation and beat-to-beat instability of the surface ECG in patients with HF. Numerous studies have identified key changes in the early and late phases of repolarization in cardiac myocytes from failing hearts [35, 46]. These include down-regulation of repolarizing potassium (K) currents (I_{to} , I_{Kr} , I_{Ks} , and I_{K1}), an increase in late Na current (I_{Na}) density [66], as well as defective intracellular calcium (Ca^{2+}) cycling [8, 9]. Notably, HF results in impaired sequestration of Ca^{2+} by the sarcoplasmic reticulum (SR) Ca^{2+} ATPase (SERCA2a) and concomitant up-regulation in Ca^{2+} extrusion by the electrogenic Na^{+} - Ca^{2+} exchanger (NCX), which generates a net inward depolarizing current during the plateau phase of the action potential [7, 46].

Excessive action potential prolongation in HF facilitates the formation of arrhythmogenic early afterdepolarizations (EADs) [11, 69]. The ionic mechanism involves the recovery of I_{Ca-L} from inactivation during the prolonged duration of the action potential [30, 43]. This, in turn, results in the voltage-dependent reactivation of I_{Ca-L} at a time when membrane resistance is high (plateau phase). The inward current generated by I_{Ca-L} reactivation results in a secondary membrane depolarization that interrupts the repolarization phase of the previous beat. If large enough, this secondary premature depolarization can reach the

threshold required to propagate to downstream cells forming a premature beat. EADs can be self-serving in that they act to further prolong APD, and therefore facilitate the reactivation of I_{Ca-L} causing more EADs. Importantly, EAD-mediated premature beats that arise during the so-called “vulnerable window” of repolarization (roughly the time interval between the peak and end of the T wave) have the potential to initiate sustained arrhythmias via a reentrant mechanism [62]. For this to happen, an appropriate electrophysiological substrate that facilitates unidirectional conduction block of the premature impulse is required [62]. The substrate could involve electrophysiological changes in the gradients of repolarization and conduction as well as structural discontinuities (i.e. fibrosis, scar), all of which are present in the failing heart. We and others have highlighted the importance of heterogeneous APD prolongation across the ventricular wall in the formation of an arrhythmogenic electrophysiological substrate [2]. Specifically, we found that the pronounced sensitivity of mid-myocardial and endocardial muscle layers to APD prolongation in HF facilitated intramural conduction block at the interface between the subepicardium and midmyocardium where the largest spatial gradient of repolarization existed [2, 50]. This was followed by the genesis of sustained transmural reentrant circuits that supported the early maintenance of VT. The mechanism underlying increased transmural repolarization heterogeneity in HF has been attributed, at least in part, to reduced cell-to-cell coupling which suppresses the homogenizing force of electrotonic interactions between adjacent muscle layers [50].

Given the importance of APD prolongation to the formation of EAD-mediated triggers and the arrhythmogenic substrate, strategies aimed at normalizing APD in HF are expected to exert protective electrophysiological effects. Unfortunately, available pharmacological agents that shorten APD, such as calcium channel blockers, are not ideal in certain situations because of their confounding effects on contraction in a patient population that often requires inotropic support [24]. As such, novel gene therapy strategies designed to reverse excessive APD prolongation without impairing contractility are warranted. To date, such strategies have not been directly applied to suppress arrhythmias in the failing heart. However, in a proof-of-principal study, Lebeche et al [41] found that adenoviral-mediated overexpression of Kv4.x, the gene encoding I_{to} , prevented pressure overload-induced APD prolongation and, in doing so, abrogated the hypertrophic response via a calcineurin-mediated pathway [41]. While I_{to} does not play a primary role in controlling terminal repolarization in humans or large animals, it sets the plateau voltage at which all subsequent currents are activated. Thus, it will be critical to evaluate the impact of this strategy in more clinically relevant animal models. A major issue to be resolved is the effect of I_{to} overexpression on the activation profile of I_{Ca-L} which may act to prolong rather than shorten APD. Moreover, I_{to} is heterogeneously expressed across the ventricular wall with epicardial cells exhibiting the greatest magnitude [6]. It will be important to evaluate whether these ionic heterogeneities, which have been implicated in arrhythmic disorders such as the Brugada Syndrome [5], are elevated or reduced following gene transfer of Kv4.3. Nonetheless, the findings of Lebeche and colleagues imply that K channel remodeling may not simply be a consequence of HF but rather a cause for it. This argues in strong favor of pursuing APD reducing strategies perhaps as a means of hindering disease progression. Whether gene based approaches aimed at overexpressing repolarizing K

channels can be used to reverse remodel the failing heart following onset of LV dysfunction is yet to be determined.

In another study, Mazhari *et al* [44] used gene transfer to increase the activity of an accessory K channel subunit in a model of prolonged QT interval. By ectopically expressing KCNE3, an important regulatory component of the delayed rectifier K channel, they were able to accelerate cardiac repolarization and abbreviate the QT interval [44]. Of note, these authors demonstrated using computer modeling that gene delivery strategies targeting K channels could in theory exacerbate arrhythmias if they were to promote repolarization heterogeneity across the transmural wall, reaffirming our earlier findings regarding the functional significance of these heterogeneities [2].

ALETERD CONDUCTION IN HEART FAILURE

Unidirectional conduction block is a prerequisite for reentrant arrhythmias and conduction slowing is typically a key predisposing factor for conduction block. Therefore, a better understanding of conduction abnormalities in the failing heart is likely to improve our ability to prevent lethal arrhythmias. Mechanisms underlying myocardial conduction slowing include reduced myocyte excitability as well as changes in intra-, extra-, and inter-cellular resistivities [3]. Numerous studies have documented the importance of changes in the expression, phosphorylation, and localization of the main ventricular gap junction protein, Cx43 in HF [1, 3, 49]. Owing to the importance of Cx43 in the regulation of cell-to-cell coupling, interventions targeting Cx43 expression and/or function may have profound implications to the treatment of a wide variety of cardiac disorders, including HF [37]. In a preclinical porcine model of post- MI remodeling caused by transient left anterior descending coronary artery (LAD) occlusion, Donahue and colleagues elegantly demonstrated that adenoviral mediated gene delivery of Cx43 but not β Gal markedly improved conduction velocity and reduced the susceptibility of animals to VT [26]. These findings highlight the importance of Cx43 gene delivery as a potential therapeutic strategy for post-MI arrhythmias [26]. Before this strategy can be translated further, however, important safety questions must be answered. For one, loss of Cx43 in response to ischemic injury is known to play a protective role, as it hinders the spread of inflammatory mediators. Indeed, Kanno *et al* [36] elegantly demonstrated that Cx43 is a major determinant of MI size. Specifically, they found that Cx43 deficient mice exhibited smaller infarcts in response to coronary occlusion compared to their wildtype counterparts [36]. These authors appropriately concluded that therapies designed to suppress arrhythmias by enhancing inter-cellular communication could ultimately lead to larger infarcts [36], and by extension greater remodeling and more arrhythmias.

In addition to Cx43 expression, post-translational modifications of the protein (namely by phosphorylation) are critical in maintaining proper cell-to-cell coupling [39]. It remains to be determined whether exogenously introduced Cx43 via gene transfer will maintain a proper phosphorylation state and will exhibit effective forward trafficking, insertion, and stability at the intercalated disk where a macromolecular complex of proteins form functional gap junctions. As we gain a more comprehensive understanding of the structure-function relationships that govern gap junction communication, we will be able to custom

design Cx43 mutants or partner molecules to optimize gene based therapies for specific applications, including the different etiologies of HF [37].

REENTRANT ARRHYTHMIAS IN HEART FAILURE

The majority of clinical arrhythmias are maintained by reentrant circuits, either around an anatomical scar (anatomical reentry) or a zone of refractory tissue (functional reentry) [29]. For reentry to occur, the cardiac wavelength (λ) of the reentrant circuit or the spatial extent of the refractory tail must be shorter than the path-length around which the wavefront circulates. As such, strategies aimed at extending λ in the critical zone where reentrant circuits anchor (i.e. MI border zone) would be expected to ameliorate the incidence of arrhythmias. Theoretically, this can be achieved by hastening conduction velocity (extending the wave head) or prolonging local refractoriness (extending the wave tail). In either case, head-tail interactions would destabilize the circuit and extinguish reentry. To that end, Donahue and colleagues published a landmark study in which they developed a porcine model of inducible VT originating in the region bordering the healed MI scar. They found that gene transfer of a mutant form (G628S) of the KCNH2 gene which acts as a dominant negative suppressor of the rapidly activating component of the delayed rectifier K current exclusively to the MI border zone region resulted in local prolongation of refractoriness [59]. This, in turn, suppressed the incidence of VT presumably by extinguishing the reentrant circuit around the scar [59]. This elegant study highlights a major advantage of gene therapy over existing pharmacological agents that prolong refractoriness and that are known to promote rather than suppress arrhythmias (i.e. some class III drugs) [28]. Specially, the ability to limit the effect of the therapeutic intervention to precisely the region that requires modification (i.e. infarct border) was a key factor for the success of the gene therapy strategy [59]. Finally, these findings highlight the importance of uncovering the tissue level electrophysiological mechanisms that underlie an arrhythmia before gene therapies can be successfully applied. Unlike non-ischemic dilated cardiomyopathy in which efforts to shorten and homogenize APD are warranted [2], in the case of post-MI arrhythmias local prolongation of refractoriness was shown to be effective in eradicating the reentrant rhythm. By mapping the reentrant circuit and defining the critical isthmus, one can envision using gene delivery strategies targeted exclusively to the abnormal zones.

λ is mathematically defined as the product of conduction velocity and the effective refractory period. We evaluated the impact of conduction slowing in HF on arrhythmogenesis by measuring a correlate of λ during premature stimulation of the heart [3]. These measurements revealed preferential reduction of λ in failing compared to normal hearts, facilitating the induction of sustained episodes of VT once a critical λ was reached. Underlying the presence of shorter λ in the failing heart was its ability to support slower propagation more safely, which is consistent with previous data from computer simulations [63] and myocyte monolayers [38]. Indeed, these studies showed slower but safer conduction because of a diminished downstream non-excitatory current sink when cell-to-cell coupling is reduced. The enhanced ability of the failing heart to support slow conduction promotes λ shortening below critical levels that are required for the maintenance of stable reentrant circuits.

Since λ can be effectively modulated by improving conduction, strategies aimed at increasing N_a may be important. In elegant proof of principle studies, Protas et al [51] showed that overexpression of the skeletal but not cardiac isoform of the sodium channel was effective in preserving conduction and reducing *in vitro* arrhythmias that arise in the setting of partial membrane depolarization (i.e. simulating ischemic injury). Of note, the skeletal muscle isoform exhibits a depolarizing shift inactivation and a more favorable recovery from inactivation rendering it more resistant to ischemia compared to its cardiac counterpart. The utility of the strategy was later extended to a clinically relevant model of arrhythmias arising at the MI border zone [40]. The studies by Protas et al [51] and Lau et al [40] emphasize the notion that delivery of non-cardiac genes may be desirable therapeutically in certain conditions in which the native cardiac isoforms are adversely affected by the cellular milieu.

ALTERED INTRACELLULAR CALCIUM CYCLING IN HEART FAILURE

Malfunction of multiple calcium cycling proteins results in electromechanical dysfunction and arrhythmias in the failing heart. In what follows, we described various strategies designed to ameliorate HF-related arrhythmias by altering calcium influx through the L-type calcium channel and reuptake into the SR via SERCA2a. Our intention is not to comprehensively review all the laudable efforts in this area, but rather to illustrate the rationale for targeting defective calcium cycling as an anti-arrhythmic strategy in HF.

L-TYPE Ca^{2+} CHANNELS

Calcium entry through I_{Ca-L} correlates with the amount of Ca^{2+} that is released from the SR (excitation-contraction coupling gain). The density of I_{Ca-L} is dictated in part by the stage of HF [12, 13], as it is increased in mild-to-moderate hypertrophy, and decreased in more advanced stages of hypertrophy and failure [27, 53]. Importantly, myocytes from failing hearts exhibit attenuated augmentation of I_{Ca-L} in response to β -adrenergic stimulation [48]. Finally, considerable slowing of I_{Ca-L} inactivation in HF alters Ca^{2+} handling and prolongs APD [54]. On the other hand, HF-induced AP prolongation predisposes to I_{Ca-L} reactivation underlying the generation of arrhythmogenic EADs (discussed above).

The pore-forming α_{1c} subunit of I_{Ca-L} is regulated by accessory components that affect channel trafficking, current density and kinetics. Recently, *in vitro* and *in vivo* knockdown of the L-type Ca^{2+} channel subunit, using a short hairpin RNA template sequence, effectively reduced I_{Ca-L} and attenuated the hypertrophic response, without compromising systolic performance [14]. Strategies designed to genetically modulate Ca^{2+} channel subunits selectively in the heart could, therefore, represent a novel and useful therapeutic strategy that complements existing calcium channel blockers. Moreover, as conduction around the core of reentrant “spiral waves” is driven by Ca^{2+} , blocking I_{Ca-L} might convert or at least slow down these arrhythmias [58].

SARCOPLASMIC RETICULUM Ca^{2+} -PUMP

Both the amplitude and decay rate of the intracellular Ca^{2+} transient are blunted in cells and tissues from failing hearts [67]. These changes result from defective sequestration of Ca^{2+}

by the SR. This important deficit is caused by a reduction in the expression and function of SERCA2a in HF [46]. As such, increasing the expression and activity of SERCA2a could indeed be clinically beneficial. Pharmacological stimulation of the pump has been tested experimentally and shown to enhance mechanical function [47]. Furthermore, studies using adenoviral-mediated gene transfer in both animal models and in isolated myocytes from failing human hearts, have demonstrated the potential for restoring impaired intracellular Ca^{2+} handling and normalizing contractile dysfunction [19]. The potential safety of this approach has been confirmed in experiments that demonstrated improved contractility at no metabolic cost (i.e. cost of oxygen) in normal and failing hearts [55-57]. Targeted gene transfer techniques to increase the expression levels of SERCA2a using adeno-associated viral (AAV) vectors have also been developed, and multicenter trials in humans have been completed [31, 32, 70]. The Calcium Up-Regulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) trial was designed to evaluate the safety profile and biological effects of SERCA2a gene transfer using AAV serotype 1 (AAV1.SERCA2a) in patients with advanced HF [31, 32]. Participants in this trial were administered a single intracoronary infusion of AAV1.SERCA2a in an open-label approach. Four cohorts of patients received a single sequentially escalating dose of AAV1.SERCA2a. 12-month follow-up of patients revealed an acceptable safety profile as well as improved clinical status, reflected by symptomatic, functional, biomarker, and LV remodeling parameters [31, 32]. In a follow-up phase 2 trial, 39 patients with advanced HF were randomized to receive intracoronary AAV1.SERCA2a gene delivery at low (6×10^{11} DRP), intermediate (3×10^{12} DRP), and high (1×10^{13} DRP) doses versus placebo [31, 32]. Patient symptoms (NYHA class, Minnesota Living With Heart Failure Questionnaire MLWHFQ), functional status (6 min walk test 6MWT and $\text{VO}_{2\text{max}}$), NT-proBNP levels and echocardiographic indices were evaluated over a 6 month period. AAV1.SERCA2a treated patients exhibited improvement or stabilization in NYHA class, MLWHFQ, 6MWT, $\text{VO}_{2\text{max}}$, NT-proBNP levels, and LV end-systolic volume compared to their placebo treated counterparts [31, 32]. Significant delays in the time to adjudicated cardiovascular events, and a decreased frequency of these events were observed in all patients receiving AAV1.SERCA2a. Finally, AAV1.SERCA2a treated patients did not exhibit an increase in adverse events or laboratory abnormalities compared to placebo counterparts [31, 32].

Despite these highly encouraging findings, it remained unclear whether accelerating SR Ca^{2+} cycling using gene therapy could alter the electrophysiological substrate in a manner that would be anti-arrhythmic, an area of major interest considering the general dogma that SR Ca^{2+} overload may exacerbate SR Ca^{2+} leak through RYR2. Indeed, cAMP-dependent PKA phosphorylation which hastens Ca^{2+} cycling reduces the threshold for Ca^{2+} sparks by enhancing the response of RYR2 to luminal Ca^{2+} . Contrary to that view, however, most recent experimental evidence obtained in SERCA2a overexpressing hearts has been consistent with protection against, not promotion of, arrhythmias [16-18, 20, 42, 52]. In both rat and pig models of ischemia-reperfusion, overexpression of SERCA2a by adenoviral gene transfer (in a preventive fashion) reduced ventricular arrhythmias during the reperfusion phase. Interestingly, in the pig model where complete occlusion of coronary flow was instituted, SERCA2a overexpression did not alter the incidence of ventricular arrhythmias during the occlusion. In a rat model of post-MI heart failure, SERCA2a gene therapy

stabilized SR Ca^{2+} load, reduced RyR2 phosphorylation, and decreased SR Ca^{2+} leak [42]. These data suggest that SERCA2a gene therapy could have antiarrhythmic effects in the setting of HF potentially by reversing RYR2 leak [42]. More recently, Cutler and colleagues showed that Ca^{2+} transient alternans in ex vivo perfused guinea pig hearts with systolic HF could be significantly reduced by gene transfer of SERCA2a [17]. Blunting of alternans levels is thought to result from the normalization of Ca^{2+} transient decay kinetics. Although the study by Cutler et al [17] was performed at lower temperatures that acted to reduce the heart rate threshold for alternans, the applicability of their findings is evident. Clinically, occult microvolt T-wave alternans are used as a non-invasive risk stratification method, especially when combined with standard electrophysiology testing[15].

These experimental studies highlights important questions. Who should get serCA2a gene therapy for the prevention of ventricular arrhythmias? Should it be reserved for patients with advanced symptomatic HF such as those enrolled in CUPID or should it also be used in patients with early stage HF who have high-risk features for sudden cardiac death: significant diastolic dysfunction, LV hypertrophy, and increased microvolt T-wave alternans? In the CUPID trial, there was no evidence of pro-arrhythmia at the three-year up in the treated groups [70]. However, the treated and non-treated groups were too small to assess for subtle changes in the incidence of asymptomatic arrhythmic triggers. Indeed, clinical studies are currently underway including an international study in 250 patients, testing whether high dose AAV1.SERCA2a (1×10^{13} viral genomes) versus placebo, randomized 1:1, is an effective therapy to reduce cardiovascular events in advanced HF. This larger trial may indeed offer important new insights into the beneficial effects of SERCA2a in patients with HF, including an anti-arrhythmic efficacy.

GENE THERAPY FOR ARRHYTHMIAS IN HF: ARE WE THERE YET?

Arrhythmias in HF result from pathophysiological remodeling that occurs at multiple levels of integration, spanning the spectrum from molecular and sub-cellular changes to those at the organ-system levels. Complex alterations in a host of ion channels, Ca^{2+} -cycling proteins, and gap junction related molecules predispose the heart to arrhythmias. HF-induced ion channel dysfunction prolongs the action potential, increases spatiotemporal gradients of repolarization, promotes arrhythmogenic triggers and conduction abnormalities.

Although gene therapy was initially envisioned as a treatment strategy for inherited monogenic disorders, its potential for a much broader applicability has become increasingly apparent. Indeed, the evolution of efficient and cardiac specific gene transfer technologies, coupled with the identification of key molecular deficits in HF, have placed arrhythmias well within reach of gene-based therapies [21, 25]. Despite this, major challenges exist: the multiplicity of arrhythmia mechanisms in HF, coupled with the complex cross-talk between individual ion channels, transporters, and exchanges that are heterogeneously expressed across the heart present formidable challenges. Nonetheless, highly promising efforts for treating and/or preventing arrhythmias using targeted gene-based approaches have already been attempted. Indeed, these emerging therapies may ultimately replace existing pharmacological agents and devices that are fraught with safety concerns and practical limitations, respectively.

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