

Gene Therapy for Cardiac Arrhythmias

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Morbidity and mortality caused by cardiac arrhythmias are a major issue in developed countries. Although conventional therapeutic options including pharmacological therapy, catheter ablation, and implantable devices have shown extensive advances to help reduce morbidity and mortality, a certain segment of these arrhythmias is still refractory to treatment. Therefore, gene therapy was explored as a potential additional or alternative therapy. Gene therapy trials have been developed for bradycardia, atrial fibrillation, and ventricular tachycardia. For the treatment of bradycardia, “biological pacemaker” attempts have been examined utilizing virus vectors to eliminate inward rectifier potassium current, or to overexpress the *I_f* current to convert quiescent myocytes into spontaneously active cells. These gene therapy attempts were soon followed by gene and cell hybrid therapies, and cell transplantation therapies utilizing pacemaker cells derived from stem cells. For the treatment of tachycardia, two major strategies were conceived: 1) to increase the effective refractory period, or 2) to recover the conduction velocity. The establishment of a selective and highly efficient gene transfer method would enable us to apply these concepts into the atrial fibrillation and ventricular tachycardia models. Both concepts resulted in an elimination or reduction of tachyarrhythmias in large animal models. Although these trials proved the concept of gene therapy as an adjuvant or alternative approach for the treatment of cardiac arrhythmias, the limitation of these studies is the long-term efficacy and safety. Consequently, an improvement in the gene delivery method is required to overcome these issues.

Key Words: Atrial fibrillation • Biological pacemaker • Gene therapy • Ion channel • Ventricular tachycardia

INTRODUCTION

The cardiac conduction system in the heart works to provide an adequate propagation of electrical impulses that regulate the coordinated contraction of the atrium and ventricle. The cardiac rhythm is initially generated in the sinus node to stimulate the right atrium. After the activation of the right and left atria, the electrical activity passes through the atrioventricular (AV) node and the His-Purkinje system to acti-

vate the ventricles. Cardiac arrhythmias which disrupt this electrical conduction system fail to regulate the orchestrated cardiac contraction, and evoke several symptoms including palpitations, fatigue, and shortness of breath. Several severe types of arrhythmias induce syncope and sudden cardiac death. Sudden cardiac death is the leading cause of death in developed countries.^{1,2} Present therapeutic strategies for arrhythmias consist of pharmacological therapy, catheter ablation, and electronic devices. Antiarrhythmic drugs have the risk of causing new and lethal arrhythmic events.³⁻⁶ Recent extensive advances in catheter ablation have enabled medical practitioners to more effectively control a large percentage of cases of supraventricular tachycardia. However, the efficacy of catheter ablation is still limited in several arrhythmias including ventricular tachycardia/fibrillation and chronic atrial fibrillation.^{7,8} Implantable electronic devices can

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maintain the heart beat in bradyarrhythmias, or deliver defibrillation shocks to terminate lethal tachycardias.⁶ However, device therapies require a replacement of the device and have the risk of infection, in addition to limitations arising from their cost. Gene therapy has been highlighted as a novel therapeutic option for lethal arrhythmias that are refractory to conventional therapy, and as an alternative to replacing expensive devices during extended post-surgical patient care and maintenance.

This article discusses the development of gene therapy for treating cardiac arrhythmias. Most gene therapy approaches utilize viral vectors for gene transduction. The efficacy and difficulty of such therapy can vary depending on the area of the target region (Table 1). The improvement in the method of gene delivery to obtain a selective, homogeneous, and highly efficient gene transduction has extensively contributed to the advancement in gene therapy, especially when a large target area was involved.⁹⁻¹⁴

GENE THERAPY FOR BRADYCARDIA

The sinus node initiates the normal cardiac rhythm, and loss of this sinus node activity results in cardiac collapse. For the last several decades, electrical pacemaker devices have contributed to the rescue of numerous patients with bradycardias. Although the electrical pacemaker is well-established and a reliably working device, all patients are required to have the devices periodically replaced when the battery charge is depleted. In addition, implanting these artificial devices in patients is associated with a rare but critical risk of bacterial infection. Thus, the concept of a “biological pacemaker” has emerged as an alternative method for the treatment of bradyarrhythmias. The biological pacemaker strategy

originated with the idea of using a viral gene delivery mechanism with gene therapy, followed by the combination of gene and cell therapies.

Gene therapy

The belief that a biological pacemaker could be feasible was based on accumulated findings in basic electrophysiology. In normal hearts, ventricular myocytes are quiescent. The inward rectifier potassium current (I_{Kr}) is the primary stabilizing source for the resting membrane potential.¹⁵ The first attempt to create a biological pacemaker was to convert ventricular myocytes into pacemaker cells. The concept of this study was based on the observation that the elimination of I_{Kr} current elevates the resting membrane potential in ventricular myocytes, resulting in augmentation of the intrinsic pacemaker current. Miake et al. generated a dominant negative mutation of Kir2.1 that codes I_{Kr} channel (Kir2.1AAA). They injected an adenoviral vector containing Kir2.1AAA into a guinea-pig ventricle. Three days after the gene transduction, the guinea-pig exhibited a spontaneous ventricular rhythm.¹⁶ In a follow-up study, they also revealed that the elimination of I_{Kr} current led to a prolongation of the action potential duration (APD), in addition to an elevation of the resting membrane potential.¹⁷ Also, a genetic defect in Kir2.1 was reported as a subtype of long QT syndrome (Andersen-Tawil syndrome).¹⁸ Thus, a simple elimination of the I_{Kr} channel may have an unfavorable effect.

Different gene therapy trials have focused on the hyperpolarization-activated, cyclic nucleotide-gated (HCN) gene family. HCN channels are dominantly expressed in the sinus node in the heart to produce If current. If current, also called a “pacemaker current”, is the inward current during the diastolic phase, resulting in spontaneous rhythmic activity.^{19,20} The adenoviral gene transduction of HCN2 channel induced pacemaker ac-

Table 1. Basic concept of gene therapy and the gene delivery method

Arrhythmia	Target region for gene delivery
Bradycardia	Small area of the atrium or ventricle
Atrial fibrillation	Homogeneous global gene transfer in the atrium
OMI-VT	Localized homogeneous gene transfer in infarct scar/border zones
Cardiomyopathy/LQTS/CPVT	Homogeneous global gene transfer in the ventricle

CPVT, catecholaminergic polymorphic ventricular tachycardia; LQTS, long QT syndrome; OMI-VT, ventricular tachycardia related to an old myocardial infarction.

tivity in the atrium²¹ and the left bundle branch.²²

These initial trials revealed that a gene transfer into cardiomyocytes evoked spontaneous activity, but the heart rate as a result of these biological pacemakers was relatively low. The optimal biological pacemaker has phenotypes with: 1) an adequate basal beating rate similar to normal sinus rhythm, and 2) appropriate heart rate response by autonomic regulation. Therefore, combination strategies were applied with HCN2 and Kir2.1AAA²³ or HCN2 and adenylyl cyclase 1,²⁴ which would be the optimal pacemaker. A recent trial reported that a combination delivery of HCN2 and the skeletal muscle sodium channel 1 (SkM1) transduced in the canine left bundle branch achieved a sufficiently high basic heart rate and autonomic response.²⁵

Since HCN channels are expressed in ventricular myocytes, the overexpression of HCN genes into myocytes may lead to unexpected arrhythmias. To avoid this confounding effect of HCN, another approach was invented using a "synthetic pacemaker channel". Kashiwakura et al. generated pacemaker channels from the depolarization-activated potassium-selective channel, Kv1.4, by site-directed mutagenesis.²⁶ A triple-point mutation in Kv1.4 (R447N, L448A, and R453I) converted Kv1.4 channel to a hyperpolarization-activated non-selective channel like HCN. Introduction of this synthetic pacemaker channel generated a biological pacemaker in the guinea-pig ventricle. However, the response to regulation by catecholamines may be subtle in comparison to the HCN overexpression strategy.

These trials manipulated one or two genes to make pacemaker currents. However, the characteristics of sinus nodal cells also differed in other channels.²⁷ Thus, the approach to "reprogramming" sinus nodal cells from cardiomyocytes was explored. This novel strategy tried to convert cardiomyocytes into pacemaker cells by introducing a transcription factor. Based on the findings that several transcriptional factors regulate the differentiation of sinus node cells,²⁸ Kapoor et al. introduced Tbx18 into the neonatal rat ventricular myocytes, which converted quiescent myocytes into sinus nodal cells with spontaneous activity.²⁹ They also injected adenovirus coding Tbx18 into a guinea-pig ventricle, resulting in the generation of an idioventricular rhythm. The reprogrammed pacemaker cells also expressed β -adrenergic receptor and muscarinic receptors.²⁹

Gene and cell therapy

After it was preliminarily shown that gene therapy could generate biological pacemakers, novel cell therapy trials were reported. Most of the previous gene therapy trials utilized an adenovirus. Because adenovirus has a high gene transfer efficacy, however, the critical limitation is its transient expression. Consequently, another approach was devised utilizing cells as vectors to overcome this obstacle.

Cho et al. focused their attention on a polyethylene glycol-induced membrane fusion strategy,³⁰ which was originally reported to make hybridomas.³¹ They introduced an HCN family channel (HCN1) into fibroblasts using a lentivirus to produce a stable expression of the HCN current. Then they injected HCN1-overexpressing fibroblasts into guinea-pig ventricles with polyethylene glycol, resulting in the creation of biological pacemakers. Mesenchymal stem cells (MSC) were also utilized in a similar strategy.^{32,33} The injection of HCN2-overexpressed MSC into canine ventricles developed a spontaneous rhythm. In contrast to fibroblasts, MSC expresses gap junction channels, which enable a cell to cell coupling without polyethylene glycol to be obtained.

Now the biological pacemaker trial has moved onto the regeneration of pacemaker cell from stem cells. Recent extensive developments in the understanding of embryonic stem cells (ESCs) and induced pluripotent stem cells have accelerated cell therapy for bradycardia. Since ESCs have the potential to differentiate into cardiomyocytes,^{34,35} transplantation of ESC-derived pacemaker cells induced spontaneous ectopic beats.^{36,37}

GENE THERAPY FOR ATRIAL TACHYCARDIA/FIBRILLATION

Atrial fibrillation (AF) is the most common chronic arrhythmia in clinical practice, and carries substantial mortality risks. The irregular electrical activity and reduced atrial pump function slows blood flow in the atrial appendage, followed by the production of a thrombus, which causes a systemic embolism such as a stroke. In addition, AF with a rapid ventricular rate sometimes causes tachycardia-induced cardiomyopathy.

There are two main management strategies for AF.³⁸ The first strategy involves 'rate control therapy', which

allows AF to continue, with ongoing control of the ventricular rate to avoid heart failure and tachycardia-induced cardiomyopathy. The second strategy is a 'rhythm control therapy' to restore normal sinus rhythm and prevent the recurrence of AF. Anti-coagulant therapy should be accompanied with both of those strategies.

To date, numerous antiarrhythmic drugs are used for both rate control and rhythm control therapy.³⁹ However, the effects of antiarrhythmic drugs are limited, and some of these drugs like sodium channel blockers have lethal proarrhythmic side effects in patients with reduced cardiac function.⁴⁰ Catheter ablation is also widely performed to maintain sinus rhythm, and the recurrence-free rate is now similar to pharmacological rhythm control therapy in paroxysmal AF.^{41,42} However, catheter ablation is an extensively invasive therapy, and its efficacy for persistent AF is still controversial.^{43,44} Considering the disadvantages of these conventional therapies, gene therapy attempts have been developed.

Rate control

The initial gene therapy approach undertaken to treat AF was attempted on the basis of a rate-control strategy. Donahue et al. successfully suppressed atrioventricular (AV) nodal conduction in pigs by an intracoronary infusion of an adenovirus.⁴⁵ They used an adenovirus vector to deliver a gene encoding an inhibitory G protein α subunit ($G\alpha i2$) by selective infusion into the AV nodal artery. Seven days after the virus was delivered, overexpression of $G\alpha i2$ slowed the AV nodal conduction and prolonged the AV node effective refractory period (ERP). The ventricular response rate was continuously reduced by approximately 20%. In the study, they also showed the results of a $G\alpha i2$ gene transfer were more effective than conventional rate-lowering drugs such as digoxin, diltiazem, and esmolol.⁴⁶

Since the activation of AV nodal cells is regulated by Ca currents, suppression of the L-type calcium current (I_{CaL}) in the AV node is an alternative way to reduce the ventricular response. To reduce the expression of calcium channels, Murata et al. focused on a GTP-binding protein, Gem, which inhibits the trafficking of the calcium channel α subunit to the plasma membrane.⁴⁷ They introduced an adenovirus containing Gem into the AV node in porcine hearts, resulting in around a 20% re-

duction in the ventricular response during acute AF.

Rhythm control

Another approach targeting AF termination has been attempted. Since the lethal proarrhythmic effect of antiarrhythmic drugs was caused by their effect on ventricular myocytes, it is expected that an atrial selective gene transfer can be achieved. Kikuchi et al. successfully delivered an adenovirus vector using the epicardial 'painting' method, in which they directly painted the viral solution on a porcine atrium.¹² They used an adenovirus coding dominant negative mutation of HERG (HERG-G628S), which is known as one of long QT-syndrome mutants. The HERG-G628S gene transfer robustly prolonged the atrial APD and ERP without affecting the electrophysiological properties of the ventricle. In the following study, Amit et al. represented the suppression of AF in a burst pacing-induced porcine AF model by HERG-G628S gene transfer using this method.⁴⁸ An alternative gene delivery using a hybrid technique combining a direct injection of an adenovirus and epicardial electroporation also suppressed AF by a prolongation of ERP.⁴⁹

A gene therapy trial using another strategy was recently reported. Gap junctions such as connexin40 (Cx40) and connexin43 (Cx43) are designed to be another gene target for a rhythm control strategy, based on the idea that a slowed conduction velocity would be involved in the mechanism maintaining AF.⁵⁰ Bikou et al. injected an adenovirus harboring Cx43 into a porcine atrium.⁵¹ Treatment with Cx43 prevented AF events and tachycardia-induced cardiomyopathy. In addition, Igarashi et al. showed that when the painting method was used, both a Cx40 and Cx43 gene transfer dramatically improved the atrial conduction velocity and decreased the AF occurrence in pacing-induced AF pigs.⁵²

Antithrombotic therapy

Thromboembolic events, including strokes, are the major cause of mortality in patients with AF. Since thrombomodulin is known as a major regulator of vascular thromboresistance,⁵³ Kapur et al. transferred an adenovirus vector containing human thrombomodulin to a rat atrium using the painting method.⁵⁴ Thrombomodulin gene transfer significantly increased the expression level of activated protein C, whereas the

thrombin activity was suppressed. This study suggested that a thrombomodulin gene transfer has the potential to be further developed into a novel anticoagulant therapy.

GENE THERAPY FOR VENTRICULAR TACHYCARDIA/FIBRILLATION

Myocardial infarction-related VT model

It has been reported that ventricular tachycardia (VT) in the healed phase of a myocardial infarction (MI) arises from a reentry circuit.⁵⁵ The myocardial infarct borderzone is characterized by a depolarized resting membrane potential with a slower upstroke in the action potential, resulting in a reduced conduction ve-

locity. Treatment strategies for MI-related VT attempt to do the following: either prolong the ERP, or recover the conduction velocity. We reported the possibility of gene therapy using a large animal MI-related VT model. We established a reproducible MI-related VT model using pigs,⁵⁶ and a localized highly efficient gene transfer method that achieved the gene transduction into the infarct borderzone.⁹ As mentioned previously, HERG-G628S encodes a dominant negative mutation of the HERG (KCNH2) potassium channel, to reduce the I_{Kr} currents. Additionally, gene delivery into the anteroseptal infarct borderzone obtained a prolongation of APD and ERP limited in this area. VT was eliminated 1 week after the gene transfer, and no proarrhythmic events were observed (Figures 1A, B).⁵⁷

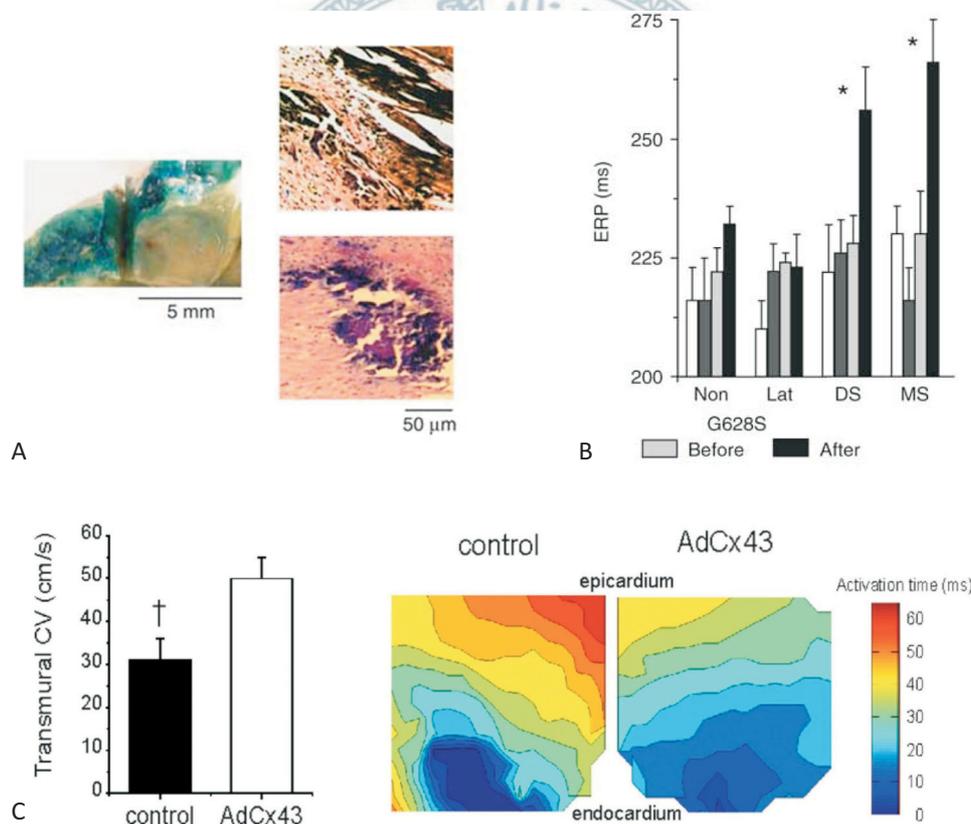


Figure 1. Gene therapy for infarct related ventricular tachycardia. (Modified from Ref. 57 by Sasano T, et al., and Ref 58. by Greener I, et al.) (A) Selective gene transfer in infarct borderzone. X-gal staining to identify lacZ gene transfer. Gross tissue shows intense blue staining, indicative of lacZ expression, in the target area at the anteroseptal borderzone in gross tissue (left panel), and in microscopic sections taken from the target region (right panel). (B) Measurement of effective refractory period (ERP) from the indicated regions. Prolongation of ERP was demonstrated in the septal border of the infarct. DS, distal anterior septal infarct border; lat, anterior lateral infarct border; MS, mid-anterior septal infarct border; Non, noninfarcted basal lateral wall. (C) Transmurals conduction velocity (CV) was faster in the Cx43-transferred group (AdCx43) than in controls (left panel). Example transmurals isochronal maps are shown in the right panel. The ventricular wedges were paced from the endocardial border. The blue coloration marks the region of earliest activation, and the red-colored area is the last activated along the transmural face of the tissue wedge. † $p < 0.05$.

An alternative approach was tested to recover the conduction velocity in the infarct area using the same animal model. For this purpose, using Cx43, a gap junction channel was delivered into the infarct borderzone.⁵⁸ The expression of Cx43 was suppressed in the infarct borderzone, and the conduction velocity was reduced at this site. An adenovirus containing Cx43 (AdCx43) was introduced into the antero-septal borderzone, which recovered the conduction velocity, resulting in a 60% reduction in the inducibility of VT (Figure 1C). In contrast to this study, Boink et al. reported that Cx32 gene transfer did not have any antiarrhythmic effect in a canine MI model.⁵⁹ In another approach to recover the conduction velocity, an adenovirus expressing SkM1 was injected into the epicardial borderzone in a canine infarct model.⁶⁰ SkM1 codes a skeletal sodium channel. The gene transduction of SkM1 increased the upstroke of the action potential, and reduced the inducibility of ventricular tachycardia/fibrillation.

Long QT syndrome

Congenital long QT syndrome is caused by mutations in several genes involved in cardiac repolarization,^{61,62} which causes sudden cardiac death with Torsade des pointes. There have been only a few trials involving gene therapy which focused on long QT syndrome. Using a transgenic mouse with a long QT phenotype (Kv1DN), a gene transfer of Kv1.5 reconstituted a 4-aminopyridine-sensitive outward K⁺ current, shortened the APD and QT interval, and eliminated early after depolarizations.^{63,64} However, arising from the limitations inherent in a study with small animals, there were few incidences of arrhythmias in this long QT model mouse. Thus, the antiarrhythmic effects should be further evaluated using another model.

Catecholaminergic polymorphic ventricular tachycardia (CPVT)

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmogenic disorder characterized by exercise- or emotion-induced polymorphic VT.⁶⁵ Most CPVTs are associated with mutations in genes encoding the ryanodine receptor (RyR2) or calsequestrin (CASQ2). CASQ2 is a calcium-binding protein located on the membrane of the sarcoplasmic reticulum. CASQ2 regulates the expression of triadin (TrD)

and junctin (JnC), and a macromolecular complex including these 3 genes controls the calcium release from the sarcoplasmic reticulum. The CASQ2 knock-out mice had a phenotype-like CPVT. A global gene transfer using an adeno-associated virus reverted the molecular and electrophysiological abnormalities in these CASQ2 knockout mice.⁶⁶ This study indicated the possibility that the global gene transfer into a whole ventricle could enable a medical practitioner to treat congenital disorders. However, further study utilizing a large animal is required.

OBSTACLES TO THE CLINICAL APPLICATION

A survey of recent experimental studies indicates that a gene transfer strategy is a hopeful way to treat arrhythmias refractory to conventional therapies. However, there are still several obstacles that remain before gene therapy can be applied to the clinical setting. The first issue is the vector to deliver the long-term expression of the delivered gene. Most of the previous studies utilized an adenovirus vector for the gene transduction due to its high gene transfer efficacy. However, the expression of a transduced gene by an adenovirus is transient. In addition, an adenovirus vector also has a risk of an immunoreaction. To overcome these issues, it is expected that better vectors with a long-term expression and higher safety will be developed. The adeno-associated virus (AAV) is known as having a longer expression and less immunological problems. Among the several serotypes of AAV, AAV9 has a higher affinity to cardiomyocytes than the other AAV serotypes,⁶⁷ It is also reported that AAV9 has a higher efficacy of gene transduction in the heart than in the other organs.

Achievement of a higher and a more stable and longer gene transfer, with lower adverse and off-target effects may help the process of gene therapy to progress closer to gaining a clinical application.

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