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Biological therapies for atrial fibrillation: ready for prime time?

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Abstract

Atrial fibrillation is a prominent cause of morbidity and mortality in developed countries. Treatment strategies center on controlling atrial rhythm or ventricular rate. The need for anticoagulation is an independent decision from the rate vs. rhythm control debate. This review discusses novel biological strategies that have potential utility in the management of atrial fibrillation. Rate controlling strategies predominately rely on G-protein gene transfer to enhance cholinergic or suppress adrenergic signaling pathways in the atrioventricular node. Calcium channel blocking gene therapy and fibrosis enhancing cell therapy have also been reported. Rhythm controlling strategies focus on disrupting reentry by enhancing conduction or suppressing repolarization. Efforts to suppress inflammation and apoptosis are also under study. Resistance to blood clot formation has been shown with thrombomodulin. These strategies are in various stages of preclinical development.

Keywords

atrial fibrillation; gene therapy; cell therapy; atrioventricular node; conduction; repolarization

Atrial fibrillation (AF) is the most common sustained arrhythmia in developed countries, affecting approximately 5 million patients in the US and having similar prevalence in Europe, developed areas of Asia, Australia and Canada.¹⁻⁶ The impact of AF on public health is substantial, with more than 400,000 hospital admissions per year and \$7 billion in healthcare costs in the US alone.⁷ The impact of AF on personal health is equally significant. The presence of AF increases stroke risk 5-fold, dementia risk 2-fold, and myocardial infarction and death risk 1.5-2-fold each.^{5,8}

A principle limitation to effective management of AF is the absence of a uniformly safe and effective therapy to restore and maintain sinus rhythm for patients with longstanding AF or those with AF in the setting of structural heart disease. In those populations, efficacy of antiarrhythmic drug therapy is limited. Within 1 year of beginning antiarrhythmic drug therapy, more than half of patients will either revert to AF or discontinue the drug for intolerable side effects (including ventricular arrhythmias, hyper- or hypothyroidism, pulmonary or hepatic toxicities, among many others).⁹⁻¹¹

Ablation has been touted as a potential cure for AF, but long-term efficacy remains elusive for patients with dilated atrial or long-standing persistent AF. In addition, broad adoption of ablation as primary therapy has been limited by a persistently high risk of procedural complications. Studies have repeatedly shown a 5-10% risk of serious complications, including pulmonary vein stenosis, atrio-esophageal fistula formation, phrenic nerve damage, cardiac perforation, stroke and death.¹² An additional concern is a recently reported 15-20% rate of “asymptomatic” cerebral embolism during AF ablation.¹³

When rhythm controlling strategies are no longer effective or are limited by unacceptable side-effects, the fallback position is to allow continued AF and focus instead on controlling the ventricular rate. The mainstay pharmacological treatments for ventricular rate control include β -adrenergic receptor blockers, calcium channel blockers, and digoxin. All are limited in some patients by side effects and/or lack of efficiency.¹⁴

Ablating the atrio-ventricular (AV) node is the ultimate rate-controlling strategy, but this renders patients permanently dependent on electronic pacemakers. The lack of an optimal therapy for AF has motivated research into innovative alternate strategies. In this review, I discuss the current state of biological therapies for rhythm or rate control in AF and for prevention of atrial clot formation and stroke in AF.

General Principles of Myocardial Gene Transfer

Basic elements common to all gene therapies include selection of a gene transfer vector and a delivery method. Other considerations, including the genetic control elements, therapeutic gene, and tissue target, are less generalizable and must be individualized to the specific application.

Vectors

Vectors are vehicles for transport of the genetic material (transgene) into the target cells. Gene delivery vectors can be divided into viral and nonviral types. Nonviral vectors are DNA plasmids with or without complexing agents to increase probability of cellular uptake (calcium phosphate, liposomes, proteins, etc.). DNA vectors are easy to manipulate and use, but the principle limitation of these vectors is their inefficient uptake into target cells. Even with the best available delivery methods, only a negligible percentage of cells (generally less than 1%) express the transgene after DNA transfection. Limited duration of expression is an additional problem with plasmid vectors. Gene expression peaks after several days and then dissipates over several weeks.^{15,16}

The increased efficiency of gene delivery with viral vectors allowed them to quickly supplant DNA as the gene transfer vehicles of choice.¹⁷ Viral vectors are essentially wild type viruses with genetic modifications to prevent viral reproduction or pathology and to insert the transgene. Adenoviruses and adeno-associated viruses (AAV) have been the most widely used and most successful vectors for myocardial applications. Both vectors can efficiently transduce cardiac myocytes.

The adenovirus is a double-stranded DNA virus with a moderately large protein coat surrounding a genome of approximately 36 kb.¹⁸ The principle advantage of adenovirus vectors is reliable and generally robust gene expression within 1-2 days of gene transfer. Other advantages of adenoviruses include ease of production and amplification and the large genome (sufficient to package almost any gene relevant to AF therapy). Disadvantages of adenoviral vectors include difficulty transiting across structural barriers given the large size of the virus, a limited duration of gene expression (approximately 3 weeks in vivo) and toxicity from the immune response to the virus. Adenoviral vectors have been used in a number of myocardial gene therapy clinical trials that have not yet shown efficacy (due in large part to the use of delivery methods that have limited delivery to target cells) but that also have not shown any detectable toxicity.¹⁹⁻²¹

AAV are a phylogenetically distinct class of viruses, related to adenoviruses in name only. AAV have a much smaller protein coat and only a 5 kb single-stranded DNA genome, consisting of two genes: rep and cap.²² The chief advantage of AAV vectors is the possibility of long term (potentially permanent) gene expression. The principle disadvantages is the 5 kb limitation on transgene size.^{23,24} AAV vectors were used in a series of heart failure clinical trials assessing the sarcoplasmic reticular calcium ATPase (SERCA2a).²⁵⁻²⁷ Efficacy ultimately proved elusive for the SERCA2a approach,²⁷ potentially due to limitations in transgene delivery or the focus on a single intervention in a very complex problem. There were no significant toxicities noted for either vector or transgene.^{25,26}

Myocardial gene delivery

Atrioventricular nodal gene delivery

Delivery of the gene transfer vector to the AV node is theoretically achievable by either myocardial injection directly into the node or intracoronary perfusion through the AV nodal artery. Unfortunately, routinely available imaging technology limits accuracy and reliability of direct injection to the node, so all literature reports of AV nodal gene transfer have used arterial perfusion methods (an example of reporter gene delivery to the AV node is shown in Figure 1a).

A number of investigators have determined that efficient gene transfer by intracoronary perfusion requires attention to three issues: (1) overcoming innate microvascular control mechanisms for broad delivery to the local capillary layer; (2) breaching the vascular endothelial barrier to access the myocyte targets; (3) optimizing local virus-myocyte interactions for the highest probability of attachment and uptake.²⁸⁻³³ They found that the best circumstances for gene transfer included maximal local vasodilation, exposure to permeability-enhancing agents (inflammatory agents, vascular endothelial growth factor, phosphodiesterase 5 inhibitors, nitric oxide or cyclic GMP donors, etc.), and perfusion with the highest tolerable virus concentration for the longest tolerable time. With attention to these details, Sasano et al. saw evidence of gene transfer in approximately half of cells in the target with antegrade perfusion of the target artery and in more than 80% of cells with simultaneous antegrade and retrograde perfusion of the artery and vein pair.³¹ Of course,

retrograde venous perfusion requires identification of the vein draining the target, which is not obvious for AV nodal delivery but is possible for other myocardial targets.

Atrial gene delivery

Gene delivery to the atria is considerably more complicated than AV nodal delivery. Size, geometry and tissue thickness limit utility of myocardial injection methods. The absence of a dedicated atrial vasculature limits intracoronary perfusion methods. To date, the only reported widespread atrial gene transfer method is epicardial gene painting.³⁴ Kikuchi et al. showed that complete transmural gene transfer could be achieved by painting a solution containing the vector, a polymerization compound, and dilute protease onto the atrial epicardium (an example of reporter gene expression after atrial painting is shown in Figure 1b). The polymerization compound (pluronic F127) causes the vector to stick to the tissue, increasing contact time and probability of gene transfer. The inclusion of trypsin in the mixture allows transmural penetration of adenoviral vectors. Unique and as yet unidentified properties of the atrial myocardium make it particularly susceptible to gene transfer by epicardial painting. The ventricles did not appear to be sensitive to gene transfer by this method.

Another reported delivery method for atrial gene transfer is direct injection into the atrial myocardium. Intramyocardial injection leads to focused, high-density gene expression, but gene delivery is limited to the tissue volume within a few millimeters of the needle track.^{35,36} In order to enhance gene transfer efficiency, electroporation has been used as an adjunct to either plasmid or virus injection. Thomas et al. and Aistrup et al. have demonstrated viability of direct myocardial injection with epicardial electroporation for gene delivery to the atria.³⁷⁻⁴⁰ In their studies, epicardial electroporation increased the efficiency from a baseline of 10% to approximately 50% in both atria. The drawbacks of the hybrid injection/electroporation approach limiting potential clinical use include possible ventricular fibrillation if the shock is not synchronized to ventricular electrical activation, the need to inject multiple times within the thin walled atria, and challenges of epicardial access (a limitation shared by the painting method).⁴¹

Rate control therapy

Gene Therapy

Transgenes reported effective for ventricular rate control in AF have for the most part targeted G protein signaling in the AV node. Donahue et al. tested the hypothesis that *in vivo* transduction of AV nodal cells with an inhibitory G protein α subunit (Gai2) would suppress AV nodal function via an adenylate cyclase/cAMP mechanism.⁴² They used adenovirus vectors for highly efficient (albeit short-term, self-limited) gene transfer, and they delivered by perfusion of the AV nodal artery. They found that overexpression of wild type Gai2 slowed AV nodal conduction and prolonged the effective refractory period of the AV node. Animals expressing the Gai2 transgene had a 20% lower ventricular rate during acutely induced AF.

In follow-up study, Bauer et al. induced persistent AF by repetitive burst pacing of the atria, and then they transduced pigs via AV nodal artery perfusion with adenoviruses carrying either wild type Gai2, a constitutively active Gai2 mutant (Q205L), or a control gene.⁴³ Wild type Gai2 only produced a statistically significant reduction in heart rate when the pigs were asleep, but the mutant Gai2-infected pigs showed a continuous 20% heart rate reduction. This improvement in ventricular rate was sufficient to reverse the tachycardia-induced heart failure inherent to the animal model. In comparison to conventional AV nodal slowing drugs (esmolol, diltiazem and digoxin), the authors found better efficacy in this model with the gene therapy.

An alternative approach for AV nodal therapy is down-regulation of the stimulatory G protein α subunit (G α s). Lugenbeil et al. were able to achieve a 20% reduction in heart rate with decreased G α s expression from RNA interference (similar to what Donahue and Bauer saw with Gai overexpression).⁴⁴ The G α s strategy may well be complementary with the Gai approach since the 2 proteins function on opposite sides of the same signaling pathway.

Cell Therapy

A cellular approach for rate control of AF was taken by Bunch et al.⁴⁵ The investigators used cell therapy to slow AV nodal conduction by increasing collagen deposition in the perinodal area, thus forming conduction barriers. They extracted fibroblasts from skin biopsies in dogs, expanded the fibroblast colonies in culture, and then injected the fibroblasts, with or without TGF β 1, into the AV nodal region of the same dogs. The intervention caused a statistically significant prolongation of the AH interval during sinus rhythm (21 ms for fibroblasts alone, 51 ms for fibroblasts with TGF β 1), and a non-significant increase in RR-interval (109 ms for fibroblasts alone, 280 ms for fibroblasts with TGF β 1) during burst pacing-induced AF.

AV nodal modification to achieve heart rate control during AF has now been shown with multiple different gene or cell-transfer strategies, demonstrating viability of the overall concept. Translation from proof-of-concept to clinical trial has not yet occurred, and does not appear to be actively under development. Limiting factors have been the absence of enthusiasm for development of a therapy that does not directly address the underlying rhythm problem, and the expense in preclinical development for a therapy perceived to have a niche indication.

Rhythm control therapy

Data from humans and a variety of animal models has shown that AF initiation seems to occur by focal mechanisms, and that AF maintenance is often due to reentry.⁴⁶ Electrical and structural remodeling occur as a result of either underlying heart disease or AF itself.⁴⁷⁻⁴⁹ Remodeling slows conduction velocity and shortens refractory period, thus stabilizing reentry. Strategies to prevent fibrillation have focused on interventions that disrupt the effects of remodeling, either by lengthening refractory period or improving electrical conduction through the tissue (Table).

To lengthen atrial refractory period, Amit et al. assessed gene transfer of a KCNH2 mutation (G628S) known to block the I_{K_r} current with dominant negative effects.⁵⁰ Amit chose this mutation because the dominant negative character would presumably allow it to function in a background of endogenous KCNH2 expression. They found that animals receiving adenoviruses encoding KCNH2-G628S by the epicardial gene painting method were resistant to atrial burst pacing-induced AF.⁵¹ The extent of APD prolongation and AF resistance correlated with gene expression. Peak physiological effects occurred during peak gene expression 3-10 days after adenovirus-mediated gene transfer, and efficacy decreased as transgene expression waned 10-21 days after transduction.

Soucek et al. performed a comparable study confirming that inhibition of KCNH2 function could prevent AF. They used the canine analogue of the KCNH2 mutation (CERG-G627S), delivered to pigs using their injection/electroporation method.³⁹ They followed pigs for 14 days after gene transfer and found prolongation of atrial refractory period and resistance to AF through their entire follow-up period.

Perlstein et al. investigated an alternative method for prolonging atrial repolarization with focal gene transfer of KCNE2-Q9E.⁵² KCNE2 is an auxiliary subunit for the I_{K_r} current, and the Q9E mutation increases sensitivity of the current to erythromycin-mediated block. Perlstein used the myocardial injection method (without electroporation) to delivery naked DNA plasmids encoding KCNE2-Q9E into a focal spot in porcine right atria. They found dose-dependent prolongation in monophasic action potential duration with clarithromycin exposure. The effects of the KCNE2-Q9E/clarithromycin strategy have only been tested during sinus rhythm, so efficacy against AF remains unknown.

To preserve conduction velocity in remodeling atria, Bikou et al. and Igarashi et al. tested connexin gene transfer in the porcine AF model. Bikou used their injection/electroporation method to deliver connexin 43 (Cx43) to pig atria, and they found that Cx43 overexpression prevented persistent AF through the 14 day observation period of their study.³⁷

Igarashi assessed atrial painting of adenoviruses encoding either connexin 40 (cx40) or Cx43. They found that atrial conduction impairment correlated with Cx43 expression, phosphorylation, and intercalated disk localization in the pig. Cx43 gene transfer reversed the conduction defect and prevented AF. Even though Cx40 expression was not altered by AF in the porcine burst atrial pacing/heart failure model, they also observed that Cx40 gene transfer could preserve conduction and prevent AF.⁵³ An important observation in the Igarashi study was the absence of conduction velocity effects for connexin overexpression in normal, sinus rhythm controls. This lack of effect on healthy tissue could indicate a reduced risk of side effects from the therapy.

A concern with the gene transfer strategies that target electrical remodeling is that atrial dilation and fibrosis may overwhelm the therapeutic effects of conduction or repolarization-focused interventions. The general concept of preventing structural remodeling, either by directly targeting AF-related structural remodeling or by addressing the underlying diseases that increase AF risk by independently causing atrial structural remodeling (e.g. heart failure, hypertension, diabetes, etc.), is a promising avenue for therapy that has been

explored in a very limited way. Trappe et al. evaluated a strategy to prevent caspase 3 activation and apoptosis. They used the injection/electroporation method in pigs, and a transgene designed to reduce caspase 3 via an RNA interference mechanism. They found that *in vivo* gene transfer with Ad-siRNA-Cas3 effectively reduced apoptosis, improved conduction velocity and delayed onset of AF, but it did not significantly alter myocardial fibrosis.³⁸

An approach specifically targeting vagally induced AF was reported by Aistrup et al.⁴⁰ They delivered genes encoding the C-terminal fragments of Gai and Gao to inhibit the interactions between endogenous G proteins and the muscarinic receptor, in effect disrupting the muscarinic signaling cascade. Aistrup delivered plasmids by the direction injection/electroporation method, and they checked AF inducibility with either vagal stimulation or carbachol administration. They found that combined Gai/Gao block had similar effects on APD when compared to Gai block alone, but combined Gai/Gao block was required to prevent AF. Aistrup's results suggested that vagally mediated AF requires more than just the APD shortening effects of vagal stimulation.

Overall, several strategies that attack underlying arrhythmia mechanisms appear successful for preventing AF. So far, published studies are proof-of-concept using short-term expression vectors (adenoviruses or plasmids). As of yet, there is no data on long-term durability of these therapies or on the ability of these therapeutic interventions to overcome atrial dilation and dysfunction driven by structural heart disease, hypertension or other factors associated with AF. Still, efficacy of the existing therapies in the setting of very aggressive AF induction protocols suggests reasonable optimism for translation. Long-term studies are necessary to understand durability of therapy and interactions between therapy and continued remodeling from structural heart disease or other co-morbidities in the target population.

Atrial thromboresistance

AF increases risk of stroke 5-fold. The mechanism is believed to be intraatrial clot formation and then embolization of the clot to the cerebral circulation. Atrial clot formation is thought to be caused by blood stasis in the hypocontractile, fibrillating atria along with a loss of thromboresistance of the atrial endocardium. Kapur et al. looked at this latter issue in the atria of rats subjected to aortic banding-induced cardiac pressure overload.⁵⁴ They described a decrease in thrombomodulin expression and increase in local thrombin generation as a function of increasing left atrial pressure. They recovered atrial thrombomodulin expression by atrial painting with adenoviruses encoding thrombomodulin and found that thrombin generation normalized in spite of continued stress of the aortic banding. These results suggest that efforts to improve atrial endothelial function may prevent strokes even if sinus rhythm is not restored. Further study is needed to understand the translational potential of these very exciting results.

Summary

The possibility for biological therapies to affect AF is real, but the field is still under development. Effective delivery methods for either AV nodal or atrial gene transfer have been reported. Ventricular rate control has been demonstrated with gene therapies targeting the G protein signaling cascade and cellular therapies inducing fibrosis. AF rhythm control has been described for transgenes that target underlying arrhythmia mechanisms, either electrical or structural remodeling. Further investigations are needed to assess long-term efficacy and toxicity for either rate or rhythm control strategies. Although future refinement in delivery and gene expression technology will likely improve results, currently available technologies have shown promising results, and it is conceivable that these could progress to early stage clinical trials within the next few years.

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Figure 1.

Gene delivery methods. **a**, Gene expression is evident in approximately 50% of AV nodal myocytes after perfusion of the AV nodal artery with vascular endothelial growth factor, nitroglycerin and a recombinant adenovirus encoding the β -galactosidase reporter gene. **b**, Complete transmural atrial gene transfer occurs after painting the epicardial surface with 20% poloxamer F127, 0.5% trypsin and the β -galactosidase encoding adenovirus. For each, the top is a tissue image and the bottom is a microscopic image of X-gal stained tissues. Blue coloration indicates active transgene expression. (with permission from (a) Donahue et al.⁴² and (b) Kikuchi et al.³⁴)

Table

Rhythm control strategies

Author	Strategy	Transgene	Model	Vector	Delivery method	Findings	Reference
Amit et al. 2010	APD prolongation	Human KCNH2-G628S	porcine AF-heart failure: persistent AF induced by 42 Hz atrial burst pacing with 2 sec on/off cycles	Ad	Epicardial painting	Increased atrial APD. Increased percentage of animals with sinus rhythm per day over 2 week period post-gene transfer (animals followed for 3 weeks post-gene transfer).	51
Soucek et al. 2012		Canine KCNH2-G627S	porcine AF-heart failure: persistent AF induced by 42 Hz atrial burst pacing with 30 sec on/off cycles	Ad	Myocardial injection and electroporation	Increased atrial APD. Delayed onset of persistent AF (defined as continuous AF on any daily recording) through post-gene transfer day 14 (last day of study).	39
Perlstein et al. 2005		Human KCNE1-Q9E	Porcine electrophysiology study. AF induction not reported.	plasmid	myocardial injection	Dose-dependent increase in atrial APD with clarithromycin administration	52
Igarashi et al. 2012	Conduction velocity recovery	GJA1, GJA5	porcine AF-heart failure: persistent AF induced by 42 Hz atrial burst pacing with 2 sec on/off cycles	Ad	Epicardial painting	no phenotype in SR animals. Recovery of conduction velocity to SR levels in AF animals. Increase in percentage of sinus rhythm per day through post-gene transfer day 7 (last day of the study) in AF animals.	53
Bikou et al. 2011		GJA1	porcine AF-heart failure: persistent AF induced by 40 Hz atrial burst pacing with 60 sec on/ 30 sec off cycles	Ad	Myocardial injection and electroporation	prevented development of persistent AF (defined as AF lasting > 48 hours) over 14 day period of study	37
Trappe et al. 2013	reduced apoptosis	CASP3 inhibitor	porcine AF-heart failure: persistent AF induced by 42 Hz atrial burst pacing with 30 sec on/10 sec off cycles	Ad	Myocardial injection and electroporation	Reduced atrial myocyte apoptosis. Reduced conduction anisotropy and longitudinal conduction velocity. Increased time to onset of persistent AF (defined as continuous AF on any daily recording).	38
Aistrup et al. 2011	disruption of cholinergic signaling pathway	Gai/Gaq competitive inhibitors	canine acute AF induced by cholinergic stimulation and atrial pacing	plasmid	Myocardial injection and electroporation	prevention of cholinergic stimulation induced APD shortening, reduction in cholinergic stimulation induced AF. Similar effects for Gai ± Gq on APD shortening, but additive effects on AF prevention.	40