HIGH SCHOOL EDITION
Journal of Student Research
Volume 11 Issue 3 (2022)

Application of Gene Therapy for Treatment of Atrial Fibrillation

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ABSTRACT

Atrial fibrillation is the most common arrhythmia globally and is associated with an increased risk for various other complications. Ablation, one of the most widely used treatments, is irreversible and can damage tissue surrounding the ablation line. Furthermore, many people with structural heart abnormalities or other conditions cannot be effectively treated. The complexity of atrial fibrillation and the uniqueness of each case make a universally effective treatment nearly impossible. The present paper describes the current treatments for atrial fibrillation and assesses the potential of gene therapy as a new clinical treatment. Further research into gene therapy could provide more targeted and effective treatments for atrial fibrillation and many other genetic conditions.

Introduction

Atrial fibrillation (AF) is one of the world's most common heart-related health conditions. Atrial fibrillation, according to the Cleveland Clinic, is "an irregular heart rhythm that begins in your heart's upper chambers (atria)" (Atrial Fibrillation, 2022). Worldwide there are upwards of 38 million cases, with trends indicating that the incidence rate will continue to increase (Lippi et al., 2020). Therefore, the development of efficient, safe, and cost-effective treatments for AF becomes more critical by the day.

Although relatively common, AF is a serious condition associated with various life-threatening complications. Common clinical AF treatments include medication, pacemakers, ablation, or electrical cardioversion. Each of these treatments has drawbacks and side effects that hinder their efficiency. Furthermore, many of these treatments are not effective for all people. For example, antiarrhythmics can cause severe side effects for people with structural deformities of the atrium or dysfunction of the sinoatrial node. According to the National Institute Of Neurological Disorders and Stroke, a person with AF is five times more likely to have a stroke than someone without it (HHS, 2022). Furthermore, AF increases the probability of heart failure and other cardiac events (Thomas, 2020).

Although relatively untested, gene therapy is a promising new treatment for atrial fibrillation. It can target specific genes contributing to AF and selectively upregulate, downregulate, or alter them. Gene therapy offers a precision that has been sorely lacking in the treatment of AF. Further research and human trials into gene therapy could potentially allow for it to become a new, commonly used clinical treatment with higher efficacy than any current treatments.

Current Treatments

Presently, clinical treatment for AF usually warrants medication, the insertion of a pacemaker, ablation, electrical cardioversion, or some combination of these treatments. However, each treatment has drawbacks and complications that prevent it from being completely effective and safe.

Medication

Drug treatments for AF and its symptoms can usually fall into one of three categories: heart rate controlling, heart rhythm controlling, or anticoagulants (American Heart Association, 2022).

Medications to control heart rate include β-blockers, calcium channel blockers, and digoxin. These treatments attempt to ease arrhythmias and reduce blood pressure and heart rate but cannot be prescribed to individuals with low blood pressure or bradycardia. Furthermore, people with Wolff-Parkinson-White, electrolyte imbalances, or myocarditis are recommended not to use digoxin as it can cause severe side effects such as arrhythmias, inflammation, or the narrowing of blood vessels (Illinois, 2018). Moreover, β-blockers are not recommended for people with asthma or other lung conditions, as they can cause bronchospasms (Sadovsky, 2003; British Heart Foundation, 2021).

Sodium and potassium channel blockers are another group of AF medication that attempt to control heart rhythm. Sodium channel blockers slow the rate of the initial depolarization of cardiac cells and could potentially help correct certain arrhythmias. However, standard sodium channel blockers such as flecainide, propafenone, and moricizine have increased the risk of sudden death in people with a history of myocardial infarction or sustained ventricular arrhythmias (Klabunde, 2011). Another risk with sodium channel blockers is possible toxicity from accidental over prescription that can cause severe side effects such as cardiogenic shock, cardiovascular collapse, respiratory depression, status epilepticus, and even death (Dokken and Fairley, 2022). Potassium channel blockers work by slowing the rate of repolarization, and by elongating the effective refractory period, they can help treat arrhythmias like Short QT Syndrome. Potassium channel blockers are contraindicated for people with moderate to severe kidney problems, as the medication can build up in the body and cause a seizure (Sreenivas, 2022).

Although not used alone, anticoagulants are commonly prescribed, in conjunction with other drugs, to patients with AF. Anticoagulants do not act on the arrhythmia itself but rather attempt to treat blood clots, one of the most severe complications of AF. The blood of people with AF could pool due to abnormal flow and form clots. These clots could travel to other body parts, causing a life-threatening stroke or internal bleeding. However, people with stomach ulcers or high blood pressure, who are at risk for internal bleeding, are recommended not to take anticoagulants. Furthermore, these medications increase the risk of significant bleeding from minor injuries due to their mechanism of action.

Overall, each medication has significant side effects, risks, and contraindications that prevent them from being ideal treatments for AF.

Pacemakers

Pacemakers are small devices implanted in the body using electrical impulses to correct arrhythmias and regulate heart rate. Pacemakers come in various forms, including leadless, single-chamber, dual-chamber, or biventricular pacemakers (Chen, 2022). Leadless pacemakers are inserted into the wall of the heart, generally by catheters, and therefore require no wiring to transfer their electrical impulses. A single-chamber pacemaker uses a single wire to attach to a chamber of the heart, while dual-chamber pacemakers use two wires to connect to two chambers of the heart. Finally, a biventricular pacemaker has three leads, one connected to the right atrium and a lead connected to each of the heart's ventricles.

A significant drawback of pacemakers is post-surgical complications. A study by researchers at Zunyi Medical University found that, although relatively rare, permanent implantations of pacemakers have a complication rate of roughly 8.06% (Jing et al., 2020). Complications can range from mild to severe, including complications such as capsular hematomas, capsular ruptures, capsular infections, and venous thrombosis (Jing et al., 2020).

Ablation

Catheter ablation was first introduced to clinical practice in the late 1960s. Although it was first designed to record information, it was soon adapted to treat AF (Ghzally, 2022). Catheter ablation uses minor burns and freezes to create scarring and disrupt abnormal electrical pathways to terminate the arrhythmia (John Hopkins Medicine, 2022).

Although the incidence of complications is very low, catheter ablation is not recommended for various people. Individuals with cardiomegaly or who have had AF for a long time are not likely to be recommended ablations as it has a lower efficacy for people with those conditions (Dallas, 2020). Furthermore, bleeding diathesis or coagulopathy is another major contraindication for ablation (Ghzally, 2022). The global prevalence of underlying bleeding disorders, approximately 25% in males and 46% of females, prevents ablation from treating a wide variety of people (Sadler, 2003). Furthermore, patients who undergo ablation have a chance of AF recurrence. Approximately 12.6% of patients who undergo ablation have a recurrence of AF within 12 months of the procedure (Poole et al., 2020).

Electrical Cardioversion

The first reported case of electrical cardioversion in the clinical setting was in the USSR's Academy of Medical Science in Moscow. Soviet scientists, Alexander Alexandrovich Vishnevsy and Boris Moiseevich Tsukerman pioneered the research of electrical cardioversion in the Vishnevsky Institute of Surgery. They utilized a DC shock to perform cardioversion for an individual suffering from AF for three years (Cakulev, 2009). Vishnevsky and Tsukerman continued to treat 20 more people with direct current cardioversion in 1960 (Vishnevskii and Tsukerma, 1961). Although electrical cardioversion can be an effective treatment for many people, it has been known to cause complications. A study by researchers at Turku University Hospital found that roughly 0.822% of patients developed bradyarrhythmia, and 0.665% of patients developed asystole, some needing resuscitation measures to be performed as a result (Grönberg et al., 2013). Although the risk of complications is relatively low, electrical cardioversion cannot effectively treat individuals with incessant arrhythmias (Beinart, 2021).

Although more effective than monotherapy, combination treatments are still far from ideal. The most common polytherapy for AF, a combination of catheter ablation and antiarrhythmic drug therapy, was ineffective for 29% of people, according to a study at the Hospital of the University of Pennsylvania (Starek et al., 2015). Therefore, a significant proportion of people with AF cannot be treated effectively with polytherapy. The lack of effective treatments for AF sheds light on the importance of investing in new treatment options, such as gene therapy.

Gene Therapy

In recent years there have been leaps and bounds in the understanding of genetics. With it, a new form of treatment, known as gene therapy has emerged. It entails using a vector to deliver genetic material, proteins, or nucleases to target cells to help alleviate or cure genetic illnesses.

Delivery Mechanisms

Currently, researchers are considering four main gene delivery vectors for gene therapy. These vectors are adenoviruses, lentiviruses, lipid nanoparticles, and adeno-associated viruses (Summary: Table 1).

Adenoviruses

Adenoviruses were first discovered in 1953 by Wallace Prescott Rowe and his colleagues (Wikimedia Foundation, 2022). The virus was isolated from an adenoid tissue cell culture which served as inspiration for its name. Adenoviruses are very efficient gene vectors as they can insert genetic material into dividing and non-dividing cells. Furthermore, they have a packaging capacity of approximately 35kb (kilobases)(Carver et al., 2021) and have high gene expression levels in the cells they infect. However, adenoviruses have various drawbacks that limit their use as effective vectors. Adenovirus vectors cannot integrate into a host genome; therefore, their gene expression is transient (Mitani and Kubo, 2002). Furthermore, adenoviruses are strongly immunogenic (Wold and Toth, 2013) as their surface antigens can trigger a robust immune response. This immunogenicity limits the efficacy and safety of treatment utilizing adenovirus vectors as the severe immune response could potentially destroy infected target cells.

Lentiviruses

Lentiviruses were first identified in the early 1980s as the causative agent of Acquired Immunodeficiency Syndrome (Thormar, 2013). Lentiviruses are capable of inserting genetic material into both dividing and non-dividing cells. Lentivirus vectors can also integrate into the genome of a target cell which allows their gene expression to be long-lasting, reducing the need for multiple treatments. However, integration into the genome of a target cell by a lentivirus vector can result in problems. Trials have shown that lentivirus-infected cells can become cancerous from gene dysregulation due to insertional mutagenesis at the site of lentiviral genome integration (Schlimgen, 2016). Furthermore, lentiviruses have a relatively small packaging capacity of only 8kb, which could potentially limit which genes and the number of genes that can be targeted in a treatment. Finally, lentivirus vectors can potentially trigger a cytotoxic T Cell response (Nayak and Herzog, 2010), potentially resulting in the destruction of target cells that have been treated.

Lipid Nanoparticles

Research on lipid nanoparticles (LNPs) for drug and gene delivery was pioneered in the 1980s by Canadian biochemist Pieter Rutter Cullis. Cullis later helped found Acuitas Therapeutics, which developed lipid nanoparticles for use in Pfizer-BioNTech's COVID-19 vaccine (Borealis, 2021).

Lipid nanoparticles, unlike viral vectors, have no immunogenic viral proteins on their surface, which means they cannot trigger an immune response (Zhao and Huang, 2014). Furthermore, lipid nanoparticles have shown a robust ability to enter dividing and non-dividing cells. However, the lack of reverse transcriptase means that lipid nanoparticles cannot integrate into the genome of a target cell, meaning that their gene expression is transient. Furthermore, although most cells internalize the lipid nanoparticle vector, fewer than 50% express the transgene (Mark, 2003).

Adeno-Associated Viruses

Adeno-associated viruses (AAV) are replication-defective viruses belonging to the genus Dependoparovirus. They were first identified by Bob Atchison and Wallace Rowe in the 1960s. AAVs are much less immunogenic than Adenovirus vectors and therefore make an attractive candidate for gene therapy. AAV vectors have a deoxyribonucleic acid-based genome and can infect both dividing and non-dividing cells. Their ability to integrate into a target cell's genome means their gene expression is long-lasting. AAV vectors are also nonpathogenic, further increasing their viability as a vector for gene therapy (Flotte and Carter, 1995).

The main drawback of AAV vectors is their limited packaging capacity of only around 4.5kb, potentially limiting which genes could be targeted (Hunter et al., 2017).

Vector Delivery Method

A suitable delivery method of the selected vector is another essential consideration for gene therapy. Some primary delivery methods being researched include epicardial painting, epicardial injection, and intracoronary perfusion (Farraha et al., 2018).

Epicardial painting entails mixing a vector with poloxamer and trypsin. The solution is then painted on the surface of the atria. Trypsin is present in the solution as it helps break down the cardiac extracellular matrix allowing the vector to interact with the myocardium (Solomonov et al., 2016). The main drawback of epicardial painting is that it currently requires open access to the epicardial surface of the atria (Liu and Donahue, 2014).

Epicardial injection uses gauged needles to inject gene therapy vectors directly into the heart wall (Sahoo et al., 2021). Epicardial injection results in heterogeneous but high levels of transgene expression when paired with treatments, such as electroporation, that increase cell membrane permeability (Bikou et al., 2011). However, like painting, epicardial injection required open access to the epicardial surface of the atria, increasing the risk of surgical complications.

Intracoronary perfusion entails using a perfusion catheter to deliver gene vectors. Using a perfusion catheter means the procedure is minimally invasive compared to epicardial panting and injection, which require a thoracotomy. However, intracoronary perfusion cannot target tissues with the same specificity as an epicardial injection. This lack of specificity means gene vectors could travel beyond the intended tissues causing more abnormal electrical pathways. Furthermore, gene delivery efficacy is limited by the endothelial barrier separating the blood and extravascular tissues. Researchers at the Case Western Reserve University School of Medicine achieved a 78 ±6% gene transfer efficiency with adenovirus vectors in piglets but utilized VEGF, nitroglycerin, and adenosine (Sasano et al., 2007). VEGF, nitroglycerin, and adenosine have a variety of severe side effects like hypertension, syncope, and cardiac ischemia, respectively (Kamba and Mcdonald, 2007; Kim et al., 2021; Singh and McKintosh, 2022).

Table 1. Summary information for major gene therapy vectors

	Dividing	Non Dividing	Gene Capacity	Genome Integration	Genome	Biosafety
Adenovirus	Yes	Yes	~35kb	Yes	DNA	BSL-2
Lentivirus	Yes	Yes	~8kb	Yes	RNA	BSL-2
LNP	Yes	Yes	~7kb	No	DNA/RNA	N/A
AAV	Yes	Yes	~4.5kb	Yes	DNA	BSL-1

Gene Targets

Gene therapy can both insert genes as well as remove existing genes through a variety of methods. Using CRISPR/Cas9, gene therapy can simultaneously remove and replace a mutated gene with a wild type to cure genetic conditions.

Loss-of-Function Mutations

A variety of loss-of-function mutations can contribute, if not cause, AF. Genes such as KCNQ1 (Potassium Voltage-Gated Channel Subfamily Q Member 1) that make up a subunit of potassium channels in cardiac tissue can accumulate mutations and cause some potassium channels to become less efficient or possibly even nonfunctional. Mutations in genes coding for potassium channels can result in conditions such as Long QT Syndrome (SHC, 2017), which is associated with an increased risk for AF (Platonov, 2019). Gene therapy can treat AF-associated mutations by utilizing a vector to insert a functional copy of the target gene (eg. KCNQ1). Specific vectors can even insert the functional gene into the target cell genome, potentially providing the cell with a permanent copy of the functional gene.

Gain-of-Function Mutations

Although rarer than loss-of-function mutations, gain-of-function mutations in specific genes can contribute to AF. Certain mutations in genes, including but not limited to KCNQ1 or KCNE1, which form subunits of potassium channels, can shorten the action potential duration and condense the effective refractory period of cardiac cells (Zhang, 2020). Treating gain-of-function mutations can be done through a variety of methods. For example, gene editing tools can recognize gene mutations and cut them out to halt the expression of harmful gain-of-function mutations. The three main methods for gene editing are zinc finger nucleases (ZFN), Transcription Activator-Like Effector Nuclease (TALEN), and CRISPR/Cas9 (Mah and Roberts, 2022). CRISPR/Cas9 is the most commonly used gene editing tool nowadays as it is cheaper and more effective. Furthermore, CRISPR is not limited to simple gene mutations, whereas TALEN and ZFN are (Wikimedia Foundation, 2021).

Another mechanism by which genetic disorders are treated is allele-specific silencing. Allele-specific silencing can be done either post-transcriptional or in the nucleus itself. Post-transcriptional allele-specific silencing can be done utilizing RNA-induced silencing complexes (RISCs). RISC can bind and catalyze the cleavage of mRNA molecules of mutated genes (Pratt and MacRae, 2009). RISC employs guide molecules, such as siRNA (silencing RNA) and miRNA (micro RNA), to specifically target the mutant allele mRNA of a given gene. Therefore, RISC allows for the targeted inhibition of specific genes without altering the gene directly.

Allele-silencing in the nucleus can best be done by various mechanisms known as RNA-directed transcriptional gene silencing (Weinberg and Morris, 2016). A well-studied mechanism called RNA-directed DNA methylation could be valuable for silencing mutant genes in the genome (Wikimedia Foundation, 2021). By directing DNA methylation, RNA-directed DNA methylation can selectively silence mutant genes contributing to AF. However, it is crucial to note that if a patient has no wild-type (unmutated) copy of a given gene, a functional gene will need to be provided through gene therapy after the mutated gene is silenced or nullified in some way.

Vector Capacity

Some potential target genes are much larger than the packaging capacity of the currently available gene therapy vectors. To target genes exceeding the vector packaging capacity, we can utilize a method of gene splitting where a transgene is cut into multiple segments and then delivered to a target cell by multiple vectors (Tornabene and Trapani, 2020).

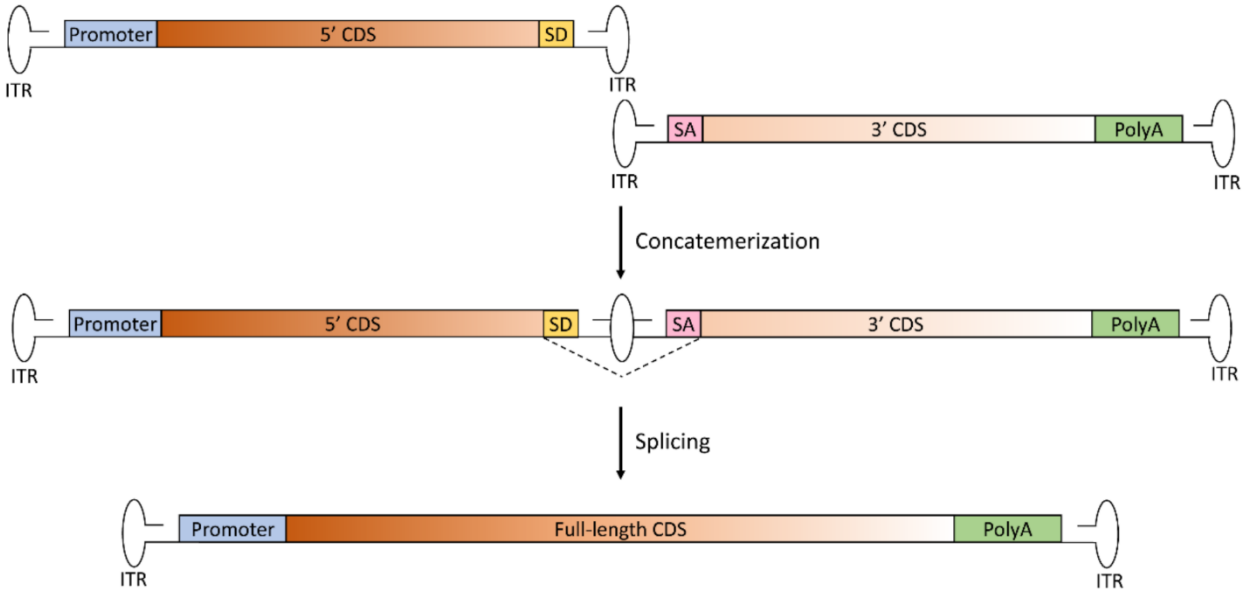

Figure 1. Gene Trans-Splicing Diagram (Trapani, 2019)

The end of each transgene segment is made into an inverted terminal repeat sequence that promotes transgene segment recombination once inside a target cell (Figure 1) (Yan et al., 2005). Inverted terminal repeats allow for multiple smaller vectors, such as adeno-associated viruses, to be used to deliver larger genes.

Conclusion

Current treatments for atrial fibrillation face efficacy difficulties that emerging genomic techniques can potentially overcome. The focus of further research on the clinical application of gene therapy can lead to a better understanding of the therapy's mechanisms and limitations. This research could pave the way for cost-effective and personalized gene therapy treatments that better account for the specific gene mutations responsible for atrial fibrillation. Furthermore, research into gene therapy for atrial fibrillation can be applied to various genetic conditions to improve the quality of life for numerous individuals.

Acknowledgements

I would like to thank Dr. James Marchant for guiding me through the process of writing this paper and providing valuable insights into cardiology and gene therapy.

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