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Introducing Genes to the Heart: All About Delivery

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Abstract

Recent clinical gene therapy trials for the treatment of heart failure have failed to meet primary efficacy endpoints and have tampered enthusiasm for the future application of cardiac gene therapy. These results have brought to light the difficulty of efficiently introducing genes into the human heart and have focused on potential problems that need to be addressed before further clinical applications. These trials however have established the safety of gene delivery vectors for cardiac targeting in humans. The sinusoidal trajectory of gene therapy continues and despite these setbacks the future of the field is promising.

Keywords

Gene Therapy; Adeno-Associated Vectors; SERCA2a: Sarcoplasmic Reticulum Calcium ATPase; Heart Failure

Recent results of cardiac gene therapy clinical trials

In the past year, the results of three recent phase II clinical gene therapy trials targeting heart failure (HF) became available[1-3]. These serial publications, however, all failed to meet primary efficacy endpoints. In the CUPID IIb trial, the efficacy of intracoronary administered recombinant adeno-associated virus (AAV) carrying sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) at a dose of 1×10^{13} DRP was examined in 250 patients[1]. In contrast to a significant reduction of clinical events in the Phase I/IIa trial, the number of adverse events in this trial was similar between the treated and the control groups. The STOP-HF trial examined the efficacy of endocardial direct injection of plasmid stem cell-derived factor (SDF)-1 with doses of 15 and 30 mg in 93 patients[2]. The primary endpoint was a composite score of a 6 minute walk distance and a quality of life questionnaire. This study again reported similar outcomes in primary endpoints between the treated and the control groups. The most recent report was the trial of adenovirus 5 mediated adenylyl cyclase 6 gene therapy using intracoronary delivery in 56 patients[3]. The primary efficacy endpoint was a combination of exercise time, echocardiography and pressure derived functional parameters before and after dobutamine challenge. Although the composite end point score was not reported, none of the parameters included in the primary

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efficacy endpoint was reported to be significant when comparing treated and control patients[3]. These results certainly frustrate the field, however, there remains some hope as the latter two trials have reported a potential benefit of gene therapy in the sub-analyses.

Why did they fail to show efficacy?

There are several possible reasons that these recent trials failed to meet the primary pre-defined clinical efficacy endpoints. These include a large placebo effect found in the control groups, diverse co-morbidities in patients that offset positive effects of gene therapy, difficulty in determining the optimal endpoints prior to initiating the trial, and insufficient power to detect the difference between the groups. Invasive procedures to deliver the genes to the heart as well as narrow patient inclusion criteria limited the enrollment of a large number of patients in the trials, which was a major limitation to address some of the above problems. However, the most convincing explanation is that the therapeutic efficacy was unfortunately not as robust as we initially expected.

Gene delivery: Critical step

For any gene therapy to work, there are two principal factors that determine the success of the therapeutic approach; gene introduction to the cells and the function of the transduced genes. Without effective gene transduction, therapeutic genes have no chance to work in the target cells. Meanwhile, even if the transduction is robust, genes with minimal or deleterious effects on cells or organs will not improve the outcomes. For the CUPID IIb trial, the most likely reason for the failure is that gene delivery vectors did not transduce the human hearts as effectively as they did in pre-clinical animal models. The viral uptake in the heart from patients who underwent cardiac transplantation only presented 20 to 561 copies of vector per mg of DNA[4]. This titer is significantly less compared to the viral uptake observed in the animal models that have consistently demonstrated therapeutic efficacy of SERCA2a gene transfer (20,000–350,000 copies of vector per mg of DNA). Thus, considering the amount of viral vector found in the heart, although only from a portion of patients, the neutral result in the CUPID IIb trial was probably due to the failure in transduction and the trial was unlikely to have examined the effects of SERCA2a gene function. For the beneficial effects of SERCA2a over-expression observed in animal models indeed translate to humans require future trials by using a more efficient gene delivery system and a much higher dose. In the other two trials, it is unclear how much gene transduction was actually achieved in their treated population, partly due to the short-term expression of plasmid and adenoviral vectors. It would be very informative if the investigators of the adenovirus 5 mediated adenylyl cyclase 6 gene therapy trial report the adenoviral titer retained in the heart of patients who underwent cardiac transplantation in the treatment arm.

Safety of vectors

One encouraging point is that none of the trials reported vector related safety issues, specifically in terms of immune responses. In the past, immune responses to adenoviral vectors have resulted in morbidities and mortalities [5]. In these trials, the vector doses were kept low and it is an important factor for the safety signal in these trials. As we envision

higher doses to enhance transduction in human hearts, immune responses will need to be monitored closely as all vectors could potentially elicit a T cell response in the heart.

How can we improve cardiac gene therapy?

In order to improve gene delivery, there are three potential strategies: 1) increasing the dose of the vectors, 2) using more efficient gene delivery methods, and 3) developing vectors with higher cardiac tropism and transduction efficacy in the myocardium.

The first strategy, increasing the dose, is the simplest approach with currently available tools. In pre-clinical studies in large animals, there is a clear dose-response relationship with the amount of administered AAV and the viral genomes in the heart. Thus, it is very likely that a higher dose of vectors can lead to increased transduction efficacy in patients as well. AAV vector doses in large animals, with similar heart weights as humans, which showed contractile improvements did not show clinical efficacy in patients. One explanation is that patients entering the trials had history of multiple procedures and interventions and in some cases extensive scarring rendering the entry of the AAV vectors more difficult. As higher doses are considered to achieve higher efficacy, an excessive amount of viral vectors can induce a cellular immune response against the vectors. In liver gene therapy studies using AAV, the magnitude of T-cell response seemed to be associated with the amount of vector administered[6] while none of the CUPID patients (where the AAV doses were much lower) had a T cell response. Although this immune reaction could be controlled by high-dose steroids, cardiac patients need to be more closely monitored for this reaction as a T-cell immune reaction in the heart might lead to fatal arrhythmias even if the reaction is mild. Thus, as clinical trials with AAVs are being planned with higher doses, patients should desirably have an implantable cardioverter defibrillator. Similarly, a high-dose of other non-viral gene delivery vectors can induce a vector-related immune response, thus careful patient monitoring is necessary after administration.

The second strategy, using a more efficient gene delivery method, has been examined in several pre-clinical large animal studies. Increased efficacy is generally accompanied by a higher invasiveness of the delivery method[7]. Retrograde coronary sinus delivery of vectors during anterior coronary artery flow blockade has been shown to achieve efficient gene transduction in pigs and is being considered as a delivery method for future clinical trials[8]. Extra-corporeal recirculating devices and closed circuit retrograde infusion during bypass result in much larger viral genomes per DNA and higher transduction efficacy overall[9]. Since majority of patients who are candidates for cardiac gene therapy have impaired cardiac function, employing invasive procedures need to be cautiously considered as it directly links to safety. Meanwhile, when patients need surgical procedures for coronary bypass or valve replacement surgeries, vector delivery using cardiopulmonary support device may be an effective approach[9].

The third strategy, employing vectors with higher transduction efficacy is being actively explored. AAV serotype 9 emerged as a vector with high cardiac tropism and has become one of the most powerful tools to target hearts in cardiac gene therapy research, especially in rodent models. This vector, thus, deserves testing in clinical trials. In addition to AAV9,

vector modification of AAVs using DNA shuffling and directed evolution have also generated efficient and more cardiotropic AAVs. A re-engineered AAV vector, AAV2i8 is a good example that transduces cardiomyocytes with high efficiency with different antigenic profiles[10]. Using this vector, we recently reported that gene transfer of constitutively active form of inhibitor 1 in a pig model of heart failure results in improved cardiac function while de-targeting the liver[11]. The altered antigen profile seems to circumvent the humoral immune reaction in humans and a broader population of patients can be treated using this vector compared to AAV1, a vector of which more than half of the candidate patients were inapplicable for the study enrollment in the CUPID trial due to pre-existing neutralizing antibodies against it. Modified mRNA and exosomes are more biological vectors that have recently emerged. By chemically modifying the nucleotide bases, modified RNA has a more stable structure and low immunogenicity in vivo[12]. It also has unique very rapid and short expression kinetics suited to over-express some of the growth factors that may cause tumorgenesis when expressed long-term. Exosomes are small cell-derived vesicles that contain proteins, RNAs, and lipids for transporting these materials to other cells[13]. As some exosomes seem to target specific cell types, these small vesicles can be used as a gene delivery vector to target the heart. These vectors essentially deliver mRNA or microRNAs directly to the cells and skip the transcriptional step, thus has the potential to improve gene transfer efficiency dramatically.

In addition to these three approaches, endogenous expression levels of target genes may be taken into consideration. That is, the same efficiency of gene transduction may result in different levels of over-expression depending on the background expression. For example, an increase in 10 copies of mRNA by gene transfer in cells with the background of 1 copy or 100 copies of basal mRNA likely leads to different effects. It is of note that in deep sequencing data of mRNA in the heart, SERCA2a (ATP2A2) mRNA numbers are relatively high even in patients with heart failure who have significantly reduced SERCA2a mRNA levels compared to normal[14]. Thus, SERCA2a may be a gene with a high hurdle and targeting genes with less mRNA presence could be an alternative approach for success with current inefficient gene delivery tools. Alternatively, development of novel promoters with very high transcriptional efficiency may overcome this issue. It is also of paramount importance that new tools and methods be tested in animal models that closely reflect human disease phenotype as comorbidities, age, and immune response likely play important roles in gene transduction.

Conclusion

By analyzing the hearts/tissues of patients who received gene therapy AAV vectors, we now realize that introducing genes into the human heart is a formidable task. There are several approaches to improve cardiac gene therapy and some of these approaches are expected to be incorporated in the upcoming clinical trials. Importantly, it is worth emphasizing that although improving transduction efficacy must be pursued, efficacy and safety need to be always balanced and safety should never be compromised in these clinical trials. Developing tools/methods for improved transduction efficacy is as important in determining the effects of therapeutic genes. More resources should be focused on improving gene delivery methods which are critical for efficiently introducing genes to the heart[15]. The many failures

experienced in clinical gene therapy trials in many monogenic diseases have been now reversed with more focused delivery and appropriate vectors. In fact, gene therapy has undergone an amazing rebirth in the treatment of monogenic diseases. The ups-and-downs we are experiencing in gene therapy for heart failure are teaching us valuable lessons and will eventually lead us to effective treatments for patients with heart failure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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