Gene Therapy in the Treatment of Heart Failure

Heart failure is a major cause of morbidity and mortality in contemporary societies. Although progress in conventional treatment modalities is making steady and incremental gains to reduce this disease burden, there remains a need to explore new and potentially therapeutic approaches. Gene therapy, for example, was initially envisioned as a treatment strategy for inherited monogenic disorders. It is now apparent that gene therapy has broader potential that also includes acquired polygenic diseases, such as heart failure. Advances in the understanding of the molecular basis of conditions such as these, together with the evolution of increasingly efficient gene transfer technology, has placed congestive heart failure within reach of gene-based therapy.

Cardiovascular gene therapy models have been among the earliest in the field, and more recently gene and cell therapies have demonstrated significant potential for the treatment of common cardiovascular problems such as refractory myocardial ischaemia and dysfunction. Accumulating preclinical studies are providing an increasingly sound scientific basis for cautious human evaluation of additional gene and cell therapy strategies. Furthermore, the anatomical compartmentalization of the heart and its accessibility by surgical and percutaneous approaches render the heart, and vasculature, a highly amenable target system for gene therapy.

As shown in FIGURE 1, myocardial dysfunction induced by genetic or specific disease states, such as coronary artery disease, hypertension, diabetes, infection, or inflammation, results in a myocardium that has a mixture of permanently lost myocytes, diseased and dysfunctional myocytes, and non-diseased myocytes. The presence of non-diseased myocytes is especially relevant in myocardial infarctions and coronary artery disease where myocardial dysfunction is patchy. However these non-diseased myocytes are under stimulation from hormonal and physical stresses that can induce apoptosis and cell death or render them dysfunctional. The targets for stem cell therapy are the permanently lost myocytes, whereas the targets for gene therapy are the dysfunctional myocytes and the prevention of the non-diseased myocytes from being lost or diseased.

In this review, we will highlight new strategies for the treatment of myocardial diseases by gene transfer to modulate cardiac function.

This review focuses on gene therapy strategies that have targeted myocardial-based diseases. Gene and vector delivery systems are presented from the perspective of designing a successful cardiovascular gene therapy protocol. The molecular targets for therapeutic intervention are then systematically reviewed according to individual pathophysiologies. These strategies are discussed mindful of the constraints of contemporary gene transfer technology and the demands imposed by the pathophysiology of interest. This is performed with the aim of instilling in the mind of the reader the likelihood of successful therapeutic intervention with any given strategy.

General Considerations

When targeting a pathophysiology for gene and cell transfer intervention, a number of factors need to be considered. These include target cell and transgene biology and the demands imposed by the pathophysiology of interest. In addition, the choice of gene and vector delivery systems and the approach to gene transfer, ex vivo or in vivo, also have a bearing on the outcome.

Targeting Cardiac Myocytes by Gene Transfer

An important question when delivering vectors to the heart is how many cardiac myocytes are needed to obtain global gene delivery. The proportion of target cells within the tissue of interest that need to be successfully gene-modified to favorably affect the pathophysiology in question is of great importance. Within this question are considerations of the efficiency of the gene transfer technology and the method of vector delivery employed. For example, the creation of a biological pacemaker requires the focal genetic modification of only a modest number of cells within the heart. In contrast, restoring myocardial contractility in the context of heart failure has a higher threshold for correction and requires the successful gene transfer to a majority of target cells.
The natural history of the target disease will have the greatest bearing on the temporal requirement of transgene expression. The required pattern of transgene expression will determine the choice of gene transfer system that can be employed for a desired outcome. For example, the strategy of transient expression of angiogenic factors by plasmid-mediated gene transfer has proven successful in relieving myocardial ischemia. In this context, transient angiogenic factor expression is sufficient to induce angiogenesis and ameliorate the target pathophysiology. Likewise, the requirement for persistent transgene expression for conditions such as heart failure is inherent in the irreversible nature of the underlying pathophysiology. Although most gene therapy applications in the heart involve a direct effect of the transgene product on the target cell, for some a therapeutic effect can be achieved in a paracrine or systemic manner. An example of the latter is therapeutic angiogenesis. In this context, the angiogenic cytokine is secreted by gene-modified cardiomyocytes and has an effect on vascular endothelial cells.

**Gene Delivery**

Gene delivery systems can be classified into two main groups, non-viral physico-chemical systems and recombinant viral systems (49, 67). The strengths of non-viral systems include the ease of vector production, reduced limitation on expression cassette size, and relatively minimal biosafety risks. The limitations include low transfection efficiency and transient effect due to intracellular degradation. The strengths of viral systems included relatively high gene transfer efficiency and, when integrating vectors are used, the capacity for long-term transgene expression. The limitations include reduced packaging capacity, inconsistencies in bioactivity and purity between vector stocks, and biosafety risks. The latter include the generation of replication competent virus, toxicity due to viral gene products, and insertional mutagenesis when integrating vectors are used.

**Non-viral vectors**

Non-viral vectors can be loosely grouped as plasmid DNA, liposome-DNA complexes (lipoplexes) and polymer-DNA complexes (polyplexes) (31). Oligonucleotides and their analogs, either alone or in complexes, are also an example of non-viral vector-mediated gene transfer. A substantial number of the human cardiovascular gene therapy protocols are based on plasmid-mediated gene transfer (43). Although myocardial plasmid-mediated gene transfer is relatively inefficient (61, 63–65) it has been the vector system on which several therapeutic angiogenesis trials have been based. In this setting, transient secretion of angiogenic factors by a modest number of gene-modified cells is sufficient for the desired phenotypic effect. Inefficient gene transfer and transient gene expression would not, however, be effective for other muscle-based cardiac pathophysiologies, the majority of which demand widespread and sustained transgene expression.

The use of synthetic oligonucleotides to modulate gene expression in biological systems has immense research and therapeutic potential (73). Therapeutic oligonucleotides take multiple forms that include antisense molecules, transcription factor decoys, catalytic oligonucleotides, and chimeroplasts. The therapeutic potential of each of these has been explored in cardiovascular disease models, and examples of these are given in the subsequent text. In addition to the generic limitations of non-viral vectors, oligonucleotides are also constrained by the problems of short biological half-life and nonspecific binding to unintended sequences.

In contrast to a relatively high in vitro gene transfer efficiency, these complexes of DNA have been found to perform less well in vivo, for targets such as vascular endothelial cells after intravascular delivery (48) and cardiomyocytes after direct myocardial injection (72). Specific limitations of this technology include poor in vivo performance, instability in serum, and rapid clearance when delivered intravascularly.

**Viral vectors**

The predominant use of viral vector systems in preclinical models of gene therapy is a reflection of the increased gene transfer efficiencies achievable with these systems. This efficiency is conferred as a result of including elements of parental virus biology that secure a favorable fate for the transferred gene. The four most developed and commonly used viral vector systems will be covered in the following discussion. Other virus-based systems do exist. Their use, however, in preclinical models is not predicted to increase in the foreseeable future due to inherent major limita-

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**FIGURE 1.** Various cell types within the myocardium of failing hearts and the targets for gene and cell therapy.
Adenoviral vectors

Recombinant human adenoviral vectors are the most commonly used vectors in preclinical gene therapy models and in clinical cardiovascular gene therapy protocols (43). The strengths of this vector system that underlie its widespread use include the relative ease of production, high functional vector titres achievable, and broad target cell tropism, particularly within the cardiovascular system.

All major cardiac cell types can be efficiently transduced by adenoviral vectors, both in vitro and in vivo. With regard to cardiomyocytes, efficient in vivo transduction has been demonstrated in gene therapy models from several mammalian species. In these models, the pattern of myocardial transduction reflects the method of vector delivery. For example, direct myocardial injection results in intense transduction at the site of injection (36), whereas intracoronary delivery results in more widespread transduction in the distribution of the injected vessel (1).

The adenovirus uses the coxsackie adenovirus receptor to integrate into the cell as shown in FIGURE 2. Beta integrins are also involved in this process of integration. In a recent study, it was shown that a decrease in the beta integrins are responsible for the decreased efficiency in adenoviral gene transfer in aging cardiac myocytes (17).

A number of maneuvers have been shown to enhance gene transfer efficiency of adenoviral vectors to the myocardium following intracoronary delivery. Physical approaches include methods that rely on catheter-based or surgically induced transcoronary pressure gradients to increase gene delivery to the myocardium (39) or the application of ultrasound energy to disperse circulating vector as it traverses the myocardium (2, 75). Chemical approaches include the use of vasodilatory and permeabilising agents that facilitate transfer of vector from the vascular lumen to the myocardium (24, 25, 66).

Target cells within cardiovascular system also include vascular endothelial and smooth muscle cells. In the presence of an intact endothelium, intravascular delivery of adenoviral vectors will result in preferential gene delivery to the endothelial cells. Smooth muscle cells, in contrast, can be transduced with moderate efficiency only after vessel injury (36).

From the preceding discussion, it can be gleaned that adenoviral vectors have been an extremely useful research tool. Translation, however, of this vector system from research to clinical applications is likely to be challenging for a number of reasons. Paramount among these is the recent and highly publicized death of a young human subject that was directly attributable to the adenoviral vector employed in this clinical trial. Adenoviral vectors have a widely appreciated capacity for evoking intense immune and inflammatory reactions, which probably contributed to the development of this significant adverse event. Similarly, in animal models, adenoviral vectors have been reported to cause myocardial and vascular inflammation, endothelial cell dysfunction, vasoproliferation, and intravascular thrombus formation. Additional constraints on the clinical use of these vectors include the preexistence of neutralizing antibodies to commonly used vectors, and the de novo development of these antibodies precludes readministration of the same vector serotype. Finally, lack of vector integration limits transgene expression and also results in dilutional loss of transgene expression in target cells undergoing division.

A number of approaches have been explored to increase the biosafety of adenoviral vectors. These include vectors with increasingly reduced amounts of parental virus genes such that current generation “gutted” adenoviral vectors are devoid of almost all of their immunogenicity. In addition, the use of adenoviral vectors with reduced fiber expression has abrogated the immune response that results in neutralizing antibodies to common adenoviral vectors.

Table 1. Comparison of major viral vector systems for cardiovascular gene transfer

<table>
<thead>
<tr>
<th>Vectors</th>
<th>Adenovirus</th>
<th>AAV</th>
<th>Lentivirus</th>
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<tbody>
<tr>
<td>Functional titer (per ml)</td>
<td>Up to $10^{12}$</td>
<td>Up to $10^{10}$</td>
<td>Up to $10^8$</td>
</tr>
<tr>
<td>Genome</td>
<td>dsDNA</td>
<td>ssDNA</td>
<td>ssRNA</td>
</tr>
<tr>
<td>Insert capacity</td>
<td>7–30 kb</td>
<td>4.8 kb</td>
<td>7–10 kb</td>
</tr>
<tr>
<td>Integration</td>
<td>No</td>
<td>Yes: chromosome 19 for wild type</td>
<td>Pseudo-random</td>
</tr>
<tr>
<td>Pattern of transgene expression</td>
<td>Transient</td>
<td>Long term</td>
<td>Long term</td>
</tr>
<tr>
<td>Cell cycle-dependent transduction</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Host/vector interactions</td>
<td>Cytotoxic and immunogenic</td>
<td>Minimally immunogenic</td>
<td>Minimally immunogenic</td>
</tr>
<tr>
<td>Clinical trial approved</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
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viral genes, further increasing their safety. The lack of viral genes makes cellular immune responses unlikely to be provoked by the vector, allowing it to persist and express transgenes in cells long term. Indeed, in a previous trial administering AAV2 expressing the transgene for factor IX intramuscularly, long-term expression (>4 years) has been demonstrated (54). However, when the same dose of the same vector [up to 1.4 × 10^{14} vg (DRP) per patient] was administered through the hepatic artery to hemophilia patients, only short-term expression of factor IX was observed (54). Finally, AAV vectors are stable at a wide range of physical and chemical conditions.

A total of 12 different AAV serotypes are known, including AAV1 through AAV12, each with different tissue tropisms (12). AAV1 has been shown to transduce skeletal and cardiac muscle efficiently. The pseudotype used in this trial, AAV2/1, contains the capsid sequence of AAV1 and the inverted terminal repeats (ITR) sequences of AAV2. The AAV2/1 vector was selected because AAV1 has been shown to be superior to the other known AAV serotypes in transducing cardiomyocytes. The AAV2 ITRs are incorporated because they have been used in many previous clinical trials and their safety profile is established (12). AAV vectors have been studied in hundreds of patients and in general have demonstrated an excellent safety profile (54). Recently, two novel AAV vectors, AAV8 and AAV9, have shown tremendous tropism toward cardiac tissue and therefore have significant promise for targeted therapies of myocardial diseases (42, 68, 81, 86).

Retroviral vectors

Retroviral vectors based on Moloney Murine leukaemia virus (MoMLV) were developed two decades ago, but these developments resulted in reduced production of toxic viral proteins and may extend transgene expression over time. Another approach to reduce vector-associated immunological responses is to modify vector tropism by engineering fiber coat proteins. This avoids transduction of unintended cells, such as antigen-presenting cells, and increases gene transfer efficiency to target cells.

Adeno-associated virus vectors

Recombinant adeno-associated virus (rAAV) vectors are derived from the dependant parvovirus AAV type 2 (57, 59). This vector system has a number of clinically favorable attributes, such as lack of parental agent pathogenicity and vector-related cytotoxicity, minimal immunogenicity, and the capacity for stable long-term transgene expression through genomic integration and/or stable episome maintenance (57–59). These vectors are tropic for striated muscle and can confer stable long-term transgene expression in skeletal and cardiac muscle in immunocompetent hosts (82–85). Major limitations of rAAV vector systems include the production of high-titre vector stocks of consistent purity and bioactivity, a limited packaging capacity of 4.8 kb, and the potential for preexistent neutralizing antibodies in human populations.

Adeno-associated viruses (AAV) have many characteristics that make them particularly well suited as vectors for cardiac-targeted gene transfer. First, AAV effectively transfects slowly or non-dividing cells, making them ideal vectors for gene transfer to cardiomyocytes. Second, AAV is not known to cause human disease, suggesting that the risks to patients, health care workers, and the public at large are low. Unlike adeno-viral vectors, vectors manufactured from AAV contain no foreign genes, further increasing their safety. The lack of viral genes makes cellular immune responses unlikely to be provoked by the vector, allowing it to persist and express transgenes in cells long term. Indeed, in a previous trial administering AAV2 expressing the transgene for factor IX intramuscularly, long-term expression (>4 years) has been demonstrated (54). However, when the same dose of the same vector [up to 1.4 × 10^{14} vg (DRP) per patient] was administered through the hepatic artery to hemophilia patients, only short-term expression of factor IX was observed (54). Finally, AAV vectors are stable at a wide range of physical and chemical conditions.

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decades ago. These vectors have been used widely in preclinical models and first entered human clinical trials in 1989. More recently, this vector has been used in the successful French trial of gene therapy for X-linked severe combined immunodeficiency (X-SCID) (13). The main limitations of retroviral vectors are an inability to transduce non-dividing cells and low-titre vector stocks. In keeping with the requirement for cell division, postmitotic cardiomyocytes and quiescent cell populations, such as intact vascular endothelial and smooth muscle cells, cannot be efficiently transduced by these vectors. As a result, many investigators have sought to overcome this block by inducing the in vivo proliferation in target cells or by employing an ex vivo strategy (61–65). Due to these limitations, the use of this vector system in cardiovascular models has fallen dramatically. This has occurred in conjunction with the increased use of adenoviral vectors and other integrating vectors that are capable of transducing non-dividing cells such as rAAV and lentiviral vectors.

Recently, 3 out of 10 children from the French X-SCID trial developed premalignant clonal T-cell proliferation that was directly attributable to dysregulation of a gene at the proviral integration site (37). The risk of insertional mutagenesis with integrating vector use had previously been considered minimal, a view now under revision. As a result of this development, there has been renewed interest in integrating vector design to improve biosafety. Modifications such as the use of self-inactivating vectors, the introduction of insulator sequences, and targeting of genome integration sites are potential ways of reducing the risk of insertional mutagenesis.

**Lentivirus vectors**

Lentiviral vectors, similar to MoMLV-based retroviral vectors, transduce target cells by genomic integration. In contrast to retroviral vectors, these vectors are capable of transducing mitotically quiescent cells, a property that broadens the range of target cells, particularly within the cardiovascular system. The first developed and most commonly used lentiviral vector system is based on the human immunodeficiency virus type 1 (HIV-1) (50). Concerns regarding the pathogenicity of the parental virus underly the drive to explore non-human-based lentiviral vector systems.

Vector modifications addressing biosafety concerns associated with contemporary HIV-1-derived lentiviral vectors include the deletion of all accessory protein genes from the packaging system, separation of packaging elements into multiple plasmids, and the use of a chimeric 5’-long terminal repeat (LTR) and a self-inactivating 3’-LTR in the vector plasmid (34, 35). These modifications also eliminate the risk of production of replication-competent virus through recombination and render the proviral LTRs transcriptionally inactive; a feature that reduces the probability of insertional mutagenesis caused by transcriptional activation of host-cell genes adjacent to the provirus integration site.

Modifications to the vector backbone to optimize performance include the incorporation of a central polyuridine tract (cPPT) and a posttranscriptional regulatory element (PRE) to enhance nuclear import and mRNA translation, respectively (76). These vector modifications have been reported to result in improved in vivo transduction efficiencies, including that of adult myocardium (4). Relatively high in vitro transduction rates have been similarly reported for vascular endothelial and smooth muscle cells. Direct in vivo intravascular delivery of lentiviral vectors has been reported to transduce endothelial and smooth muscle cells in uninjured vessels. These studies, however, did not include measures of vessel wall transduction efficiency.

The experimental use of this relatively new vector system in preclinical models is rapidly expanding, and its potential application for human gene therapy has not yet been explored. The strengths of this system include the ability to confer long-term stable transgene expression, to be concentrated by simple centrifugation when pseudotyped with VSV-G, and an increased packaging capacity compared with rAAV, the other commonly used integrating vector. The major limitations relate to biosafety, as described above.

**Hybrid vectors (viral/non-viral)**

It is envisioned that current gene transfer systems will be supplanted by hybrid systems that combine the simplicity and safety of non-viral systems with desirable properties of viral vectors (47). Virosomes are an example of such a hybrid system, and of these the Hemagglutinating Virus of Japan (HVJ)-liposome has been the most investigated within cardiovascular gene therapy models. This hybrid vector has been particularly useful in oligonucleotide-based gene therapy models (47). Clinical use of this vector awaits resolution of vector production issues, including stability, and the evaluation of efficacy and safety in large animal models.

**Design of the expression cassette**

The basic components of an expression cassette include promoter/enhancer elements, the gene(s) of interest, and an appropriate mRNA stabilizing polyadenylation signal. Other frequently employed cis-acting elements include internal ribosome entry site (IRES) sequences to allow expression of two or more genes without the need for an additional promoter, and introns and posttranscriptional regulatory elements to improve transgene expression. Of these, promoter selection has received the greatest attention in the literature.

Models of gene therapy generally employ expression cassettes containing strong viral promoters that
are constitutively active in a wide spectrum of cells. For several reasons, the resulting pattern of gene expression is unlikely to be optimal for most clinical applications. Spatial and temporal regulation of transgene expression can be achieved by the choice of promoter. Tissue-specific promoters can be used to restrict transgene expression to the desired target cell population and avoid unintended cells such as antigen presenting cells. For example, cardiomyocyte-specific promoters such as alpha-myosin heavy chain have been employed to restrict gene expression to the myocardium. Similarly, the smooth muscle-specific promoter SM22α has been demonstrated to restrict gene expression to cells of this type.

Promoters subject to pharmacological or physiological regulation can be used to achieve the desired temporal pattern of transgene expression. Ligand-dependent regulatory systems are the subject of intense study, but have not been widely used in cardiovascular gene therapy models (1a, 1b). Systems exploiting physiological regulation can be designed to incorporate promoters with transcriptional activity that is contingent on signals provided by the pathophysiology of interest. Hypoxia, intravascular shear stress, and left ventricular strain have all been used in models of this type of regulation.

Adeno-associated virus (AAV) vectors package single-stranded genomes and require host-cell synthesis of the complementary strand for transduction. However, when the genome is half wild-type size, AAV can package either two copies or dimeric inverted repeat DNA molecules (15, 33, 55, 56). Dimeric, or self-complementary molecules (scAAV), spontaneously re-anneal, alleviating the requirement for host-cell DNA synthesis. Samulski and colleagues at University of North Carolina engineered such vectors and found that these scAAV resulted in rapid and higher levels of transgene expression than a conventional single-stranded vector (15, 33, 55, 56). These novel scAAV vectors represent a biochemical intermediate in rAAV transduction and should provide new insights into the biology of vector transduction. However, the size of the cassette sizes have to be half of a normal size single stranded vector (i.e., 2.3 kb), which further limits the number of genes that can be used with scAAV.

Vector Delivery

Once a molecular target and gene delivery system has been chosen, the next step is to deliver the vector to the site of interest within the cardiovascular system. There a several approaches to vector delivery in rodents. Many of these are only of relevance to small animal models, whereas others rely on percutaneous techniques that are readily transferable from large animals into clinical practice. The method of vector delivery employed is mainly determined by the target pathophysiology.

**Catheter-based myocardial delivery**

Percutaneous catheter-based gene delivery to the myocardium in vivo can be achieved by the intracoronary route, by endocardial delivery, or by retro-infusion of the coronary veins. Variable transduction efficiencies have been reported after intracoronary vector delivery. This variability in transduction is due to a number of factors that include differences between animal species, biocompatibility of catheter and vector, pharmacological agents used to permeabilize vasculature, and vector-related variables such as titer. These factors need to be optimized when the target pathophysiology demands high transduction efficiencies. Viral vectors based on adenovirus, rAAV, and lentivirus have all been reported to result in gene transfer efficiencies approximating 50%. The latter two are capable of conferring long-term transgene expression. However, only the former two vectors have been shown to rescue the heart failure phenotype in genetically predisposed animals when the appropriate anti-heart failure transgene is expressed. In contrast, other pathophysiology requires only focal gene transfer for phenotypic effect. For example, gene delivery to AV node via the intracoronary route resulted in gene transfer to 50% of AV nodal cells and physiological slowing of electrical conduction (23).

In contrast, percutaneous endocardial delivery is limited to focal gene transfer to the myocardium. This method of vector delivery and pattern of gene transfer lends itself most readily to applications such as therapeutic angiogenesis (51) and to a lesser extent focal arrhythmia therapy (27). Gene delivery can be achieved using steerable needle-tip catheters through which vector may be injected to predetermined regions of endocardium. The catheter tip can be guided by fluoroscopy or by intracardiac echocardiography, both of which have been used safely in large animal models. The non-fluoroscopic electro-mechanical mapping system is even more suited to endocardial gene transfer approach. A number of specialized catheters have been developed to aid in these direct gene deliveries. They include the NOGA catheter (Johnson & Johnson) and the Stileto catheters (Boston Scientific). These systems, which can include electromechanical mapping, are capable of identifying ischemic myocardium and also guiding delivery catheters for gene transfer interventions such as therapeutic angiogenesis. In FIGURE 2, a stiletto catheter is positioned in the anterior wall of a swine ventricle, and a solution containing viral vectors is being injected. Importantly, the feasibility, safety, and potential efficacy of this approach has been established in phase 1 clinical trials of patients with medically refractory severe angina secondary to multi-vessel coronary artery disease unsuitable for revascularization by conventional means (44, 52, 79).

A novel catheter-based technique for myocardial gene delivery has recently been reported. This
approach consists of the retrograde infusion of vector via the coronary veins and can result in gene transfer efficiencies comparable with intracoronary injection (3). This approach has been validated in a porcine model of acute myocardial injury. In this study, transfer of decoy NF-κB oligonucleotides to acutely ischemic myocardium resulted in limitation of infarct size and retention of regional myocardial contractility. The ability to deliver genes to myocardium through disease-free veins while bypassing diseased arteries that are likely to impede vector delivery is a clinically attractive attribute of this vector delivery strategy.

More recently, a percutaneous and clinically applicable catheter-based gene delivery method was developed that allowed selective antegrade myocardial gene transfer with concomitant specific coronary vein blockade and a high gene expression in targeted myocardium, as shown in FIGURE 3 (40). This method has yielded significant gene transfer to the myocardium in a pig model of heart failure (40).

Pericardial delivery

Myocardial gene delivery has been attempted via the pericardial space. The rationale underlying this approach is the anatomical proximity between the pericardium and the myocardium and the accessibility of the pericardial sac for percutaneous vector delivery. Myocardial transduction by this approach has been reported to be low, with vector mostly transducing pericardial cells (32). Gene transfer efficiency, however, can be significantly improved by the co-administration of proteolytic enzymes that disrupt the pericardial cellular and extracellular barriers to the myocardium (32). An alternate strategy involves vector delivery to the pericardial space before the cell-lined pericardial sac has developed. This strategy has been tried in 5-day-old neonatal mice and has been reported to result in high transduction rates after subxiphisternal vector delivery, with acceptably low procedural mortality (16). Other investigators have taken advantage of the ability of vectors to transduce pericardial cells following intrapericardial delivery. Transgene expression from these cells can be utilized as a platform for therapeutic protein production and delivery. An example of this is the secretion and paracrine effect of angiogenic factors from gene-modified pericardial cells.

Surgical delivery

Surgical gene delivery is considered to be the most invasive approach because of the significant morbidity associated with achieving access to the myocardium. Direct injection of vector into the myocardium of an intact animal can be achieved via the transthoracic or subxiphisternal approaches. Use of this method in the literature has been restricted to small animal models (36). The usual target in this context is the myocardium or the left ventricular cavity. Injection of the latter results in not only myocardium delivery via coronary perfusion but also systemic vector spread. In certain conditions, such as hypertension, systemic delivery might be desirable. Direct myocardial vector delivery in small animals can result in moderate transduction efficiencies. In larger animals, however, the
amount of accessible myocardium is restricted and hence significantly limits gene transfer efficiency. In the context of human gene therapy, surgical vector delivery can potentially be performed at open cardiac surgery, thorascopically or during the ex vivo phase of cardiac allografting. The choice of surgical approach will largely depend on the clinical setting and the availability of noninvasive or percutaneous alternatives. All of these approaches have been successfully used in preclinical animal models of myocardial gene transfer. Of note, early phase therapeutic angiogenesis trials involving surgical protocols have been initiated in human subjects with chronic refractory angina.

“The V-Focus system enables a closed circuit to be percutaneously established between the coronary arteries and the coronary sinus.”

Gene delivery to the myocardium by multiple direct injections in open-chested small animals is a well established technique (36). This results in a pattern of moderate but multifocal transgene expression. This pattern, however, is sufficient to affect global phenotypic changes such as improved ventricular contractility and alteration of ECG intervals. Improved and more widespread myocardial transduction can be achieved using a recently described method of vector delivery (39). This method entails delivery into the aortic root via a catheter introduced into the LV cavity and advanced cranially into the aortic root, while the ascending aorta and pulmonary artery are transiently occluded. This method relies on the production of a transcoronary myocardial perfusion gradient for vector delivery. Variations on this method include avoidance of pulmonary artery occlusion, occluding the distal rather than ascending aorta, the use of hypothermia to prolong cross-clamp times and pharmacological induction of asystole. More recent developments avoid surgery and rely on balloon catheters for aortic and right-heart occlusion while maintaining the ability to deliver vector into the proximal aorta (2, 22, 38, 73a). Furthermore a similar technique has now been refined in murine models where periods of cardioplegia are instituted during the gene transfer (14, 46). The successful application of this method in small animal models has the potential to be extrapolated into the clinic within the context of cardiac surgery that requires aortic cross-clamping and cardiopulmonary bypass.

**Circulatory perfusion technique**

A novel perfusion technique that allows targeted delivery of gene therapy has been recently developed: the V-Focus system (71). The V-Focus delivery system allows for a minimally invasive, percutaneous procedure to establish isolated regional perfusion of the myocardium. The system is designed to substantially isolate the coronary circulation from the general circulation, with minimal disturbance to the existing cardiac function. The technology is intended for administration and uptake of the selected gene therapy agent almost exclusively to the myocardium. The system is designed to minimize exposure of the therapeutic agent into the systemic circulation. The only unavoidable loss from the circuit occurs via the Thebesian veins, which communicate directly with the cardiac chambers and not with the coronary sinus. In most patients, the blood flow to these vessels is <10% of the total coronary circulation. Cardiac circulation is supplied through the left and/or right coronary arteries and returned to the general systemic circulation through the coronary sinus. The V-Focus system enables a closed circuit to be percutaneously established between the coronary arteries and the coronary sinus. Through percutaneous catheters, it selectively isolates and circulates the gene transfer agent through the coronary circulation. Adequate oxygenation of the myocardium is maintained in part by circulating the perfusate through an extracorporeal membrane oxygenation system (ECMO). Once a closed circuit is established, the vector is directly injected into the circuit and circulates through the myocardium for approximately 10 min. The system allows for direct administration of a therapeutic agent to the heart while providing circulatory support to the heart during the procedure to maintain normal cardiac functioning (71). This technique has allowed widespread transduction of SERCA2a in an ovine model of heart failure with a substantial increase in contractility both with adenoviral and AAV gene transfer. The V-Focus system is composed of two standard percutaneous intervention catheters for perfusion of the right and left coronary arteries, a proprietary coronary sinus recovery catheter (CPB) components including an oxygenator with infusion manifold, a perfusion roller pump, circuit pressure monitors, and blood temperature control module.

The system circuit includes arterial perfusion lines made of standard CPB 1/4-in. tubing. The venous recovery line incorporates a port for priming of the circuit and subsequent infusion of fluid. It also has a connection for the circuit inlet pressure monitor. The arterial perfusion line includes a bubble trap and a port for administering the therapeutic agent and a connector to the collection bag, which is used to collect the perfusate following the procedure. All blood-contacting surfaces are enclosed within the system, and the remaining perfusate (within the collection bag) can be easily disposed of at the end of therapy administration using normal biohazard procedures.

**Ex vivo delivery**

Ex vivo vector delivery in the context of cardiovascular gene therapy has been reported in two different settings. The first is the delivery of vector to the whole heart before allogeneic transplantation. Intracoronary or direct myocardial vector delivery has been reported
to result in moderate transduction efficiencies in small animal models. As a result of ex vivo genetic manipulation in these models, allograft survival can be prolonged and allograft arteriopathy can be significantly reduced. Vascular smooth muscle and endothelial cells have been subject to successful ex vivo vector delivery (61). The retroviral vectors used in these early studies required target cell division for transduction, and consequently these cells could not be transduced in vivo. Recent progress in gene transfer technology has diminished the need for ex vivo transduction of these cell types. Nonetheless, an ex vivo approach remains viable, particularly within the context of the emerging technology of cell transplantation and stem cell-based cardiac regeneration. These cells can be gene-modified to confer diverse properties such as secrete recombinant proteins, reduce unwanted immune responses, prolong cell survival in vivo, and confer a capacity for exogenously controlled proliferation. In addition, cells can be manipulated to acquire specialized functions such as pacemaker capabilities or functional electromechanical coupling with host cardiomyocytes.

**Targets for Intervention**

The number of molecular targets for each pathophysiology is likely to increase with advances in the knowledge of the molecular basis of cardiovascular disease. With this in mind, the reader should be aware that the remaining part of this section is not meant to be exhaustive but rather representative of the molecular targets that have been pursued in the literature (38). These targets are grouped according to the tissue of interest and include the myocardium, the vasculature, and the cardiac conducting system.

**The myocardium**

Heart failure is a major health problem in the modern world. Despite significant gains in medical and surgical treatment of this condition, the disease burden imposed continues to increase, particularly as the population ages. Late-stage heart failure has a poor prognosis, and effective treatment is limited to transplantation and the use of mechanical assist devices. These treatment options are severely constrained due to the perennial shortage of donor hearts and the restricted availability of costly mechanical devices. Faced with these challenges, researchers have vigorously explored novel therapeutic options, including gene-based treatments. Consequently, many animal models of gene therapy for heart failure have begun to emerge (38). In the following section, a systematic overview of the more promising molecular targets is provided. In **FIGURE 4**, we summarize the different transporters and protein within the myocardial cell that have been targeted by gene transfer.

**FIGURE 4.** Excitation-contraction coupling in cardiomyocytes and the various targets for manipulation
The beta-adrenergic signaling cascade

The main role of the beta-adrenergic receptor (βAR) signaling cascade is to regulate heart rate and contractility in response to agonist catecholamines. Binding of an agonist activates the heterotrimeric G-protein second messenger system, which in the context of the cardiomyocyte results in stimulatory G-protein alpha-subunit (GαS) dissociation. This stimulates adenyl cyclase (AC) to increase cyclic adenosine monophosphate (cAMP) production, which then activates protein kinase A (PKA). The PKA-dependent phosphorylation of several downstream targets such as troponin-I, phospholamban (PLB), and L-type calcium channels mediate the physiological effects of βAR stimulation.

Chronic heart failure is associated with increased sympathetic outflow. Although initially this is compensatory, in the long term, chronic βAR stimulation contributes to worsening of the pathophysiology. A number of alterations in the βAR signaling cascade have been described in this context, and these include βAR downregulation, upregulation of βAR kinase (βARK), and increased inhibitory G-protein alpha-subunit (GαI) function (7–10, 30). Together, these alterations desensitize βARs and diminish signaling through this pathway. Several gene-based approaches have been reported to successfully restore this signaling defect and as a result rescue the heart failure phenotype. These observations were initially made in transgenic mice and then reproduced by somatic gene transfer in animal models of inherited and acquired heart failure.

Transgenic mice with augmented βAR signaling, through either βAR or βARK inhibitor (βARKct) overexpression, display increased baseline and stimulated contractility. When these mice were cross bred with genetic mouse models of heart failure, the resultant mice were rescued from the heart failure phenotype. Of note, in these cross-breeding experiments, the βARKct overexpressing mouse out performed the βAR mouse. This suggests that reversing desensitisation of βARs is mechanistically more important than just overexpressing them. Additional information gleaned from these experiments is that threshold overexpression levels exist for βARs, above which cardiomyopathy ensues. Moreover, different thresholds exist for different βARs, with β2ARs having a much lower toxicity threshold than β1ARs.

Several in vivo gene transfer strategies have evolved from the transgenic mouse experiments. For example, both β2AR and βARKct have been reported to result in augmented or rescued βAR signaling following adenovirus-mediated transfer of each gene to normal or dysfunctional rabbit cardiomyocytes, respectively. Similarly, in vivo adenovirus-mediated β2AR or βARKct gene transfer to the normal rabbit heart resulted in increased contractility, following transcoronary vector delivery. More importantly, gene transfer of the adenoviral vector encoding βARKct was reported to have beneficial effects in a rabbit myocardial infarct (MI) model. This benefit varied according to the timing of vector delivery. If vector was delivered at the time of MI, heart failure was prevented, and if delivered 3 wk after MI, at a time when heart failure is established, then βARKct gene transfer was capable of reversing this pathophysiology (74). More recently, gene transfer of βARKct in cardiac myocytes isolated from patients with congestive heart failure has shown restoration of contractile function.

Instead of reversing βAR desensitization, other genetic manipulations of the βAR signaling cascade have been pursued with the aim of bypassing these dysfunctional receptors. These approaches generally result in augmentation of cAMP-dependant cardiac contractility. Examples of these include overexpression of adenyl cyclase or the use heterologous or “designer” receptors, which couple with the stimulatory G-protein alpha-subunit with higher efficiency than desensitized βARs.

In contrast with the small animal gene transfer data, the clinical experience with pharmacological agents suggests that genetically augmenting βAR signaling may be detrimental in the setting of chronic heart failure. For example, ionotropic βAR agonists increase, whereas βAR antagonists decreased mortality in patients with heart failure, suggesting that βAR desensitization occurs as a protective mechanism against the toxic effects of chronic sympathetic discharge (70). As a result, controversy abounds in the scientific literature as to the perceived benefits and potential dangers of the gene-based approaches to augmenting βAR. The key to reconciling these discrepancies is probably in the basic science that underpins the diverse signaling and consequent physiological effects of the βAR subtypes. Until these important differences are understood, this approach to gene therapy of heart failure is best viewed with cautious optimism.

The calcium handling proteins

The intracellular handling of calcium is central to the process of excitation-contraction coupling in muscle cells. In cardiomyocytes, membrane excitation results in the entry of calcium via the L-type calcium channel. This triggers release of intracellular stores of calcium from the sarcoplasmic reticulum (SR) via the ryanodine receptor, a calcium release channel. The resulting rise in intracellular calcium causes sarcomeric shortening and muscle contraction. Conversely, muscle relaxation is initiated by a fall in intracellular calcium, a process that is largely driven by sarcoplasmic endomembrane Ca-ATPase (SERCA)-mediated reuptake of calcium into the SR. The activity of SERCA is modulated by phospholamban (PLN), a SR transmembrane protein. Unphosphorylated PLN inhibits SERCA function, whereas PKA-dependant phosphorylation relieves this inhibitory effect. Increased SERCA
activity, as a result of PLN phosphorylation, results in increased SR calcium reuptake and subsequent release. This is the mechanism by which βAR signaling, with PKA activation and PLN phosphorylation, results in increased cardiac contractility.

Several calcium-handling protein defects have been described in the context of chronic heart failure (7, 70). Of these, increasing SERCA function or decreasing the inhibitory effect of PLN has been the subject of most gene transfer approaches targeting altered calcium physiology (38). The net effect of either overexpressing SERCA2a or downregulating PLN function is to restore a favorable PLN to SERCA2a ratio, a measure that seems to be of greater physiological relevance than either change alone. Support for these approaches, like the βAR signaling strategies, comes from transgenic mice where overexpression of SERCA2a or PLN ablation has been reported to result in favorable changes in cardiac hemodynamics and to prevent dilated cardiomyopathy in cross-bred genetically predisposed mice, respectively.

In experiments targeting SERCA2a function, restoration of the calcium transient morphology in cardiomyocytes isolated from failing human hearts has been reported after adenovirus-mediated gene transfer of SERCA2a (20, 21). In vivo transcoronary gene transfer of the same adenoviral vector to pressure loaded myopathic rat hearts was also found to result in restoration of SERCA2a levels, improved cardiac hemodynamics, and increased animal survival (19, 20). Gene transfer of SERCA2a in an animal model of heart failure resulted in a restoration of the end-systolic pressure-volume relationship, a decrease in end-diastolic volume, and an increase in stroke volume. Furthermore, survival in this animal model was improved from 9% to 64% (19, 20).

In fact, gene transfer of SERCA2a has shown tremendous promise, and there are now two clinical trials in preparation using SERCA2a as a target: 1) a phase 1, randomized, double-blinded, placebo-controlled, dose escalation trial of intracoronary administration of AAV1.SERCA2a (MYDICAR) in patients with congestive heart failure (Celladon) and 2) a phase 1 study evaluating the safety and biological effects of AAV6.SERCA2a in non-ischemic patients undergoing left ventricular assist placement.

Ablation of PLN function has been achieved by either anti-sense or dominant-negative approaches. Both approaches have been validated in vitro. Of note, normalization of the calcium transient morphology and restoration of cell contractility has been reported in cardiomyocytes isolated from failing human hearts after adenovirus-mediated PLN antisense gene transfer (11). These in vitro findings have been extended in a cardiomyopathic hamster model. Improved calcium handling, cardiac hemodynamics, and retardation of the progression to heart failure were reported following transcoronary delivery of rAAV encoding a dominant-negative form of PLN (41, 45). The excitement generated by this study, which utilized clinically meaningful gene and vector delivery methods, has recently been tempered by the discovery of a dominant-negative mutation in PLN as a cause of human dilated cardiomyopathy. Additionally, inconsistencies have recently been described in the ability of PLN knockout mice to rescue heart failure in cross-bred genetic heart failure models. These latter developments raise doubts about using PLN ablation as a universal approach to treat all forms of heart failure.

An additional critical target in the calcium cycling proteins involves inhibitor-1. Two main classes of serine/threonine phosphatases, referred to as types 1 and 2, have been shown to regulate cardiac contractile performance. The type 1 phosphatase (PP1) has been implicated in the regulation of the beta-agonist responses. This enzyme is localized to SR membranes and is regulated by two heat and acid stable proteins, inhibitor-1 and -2. Inhibitor-1 (I-1) becomes active on phosphorylation on threonine-35 by PKA (11, 28). This results in inhibition of PP1 and therefore enhanced PKA-mediated protein phosphorylation, leading to amplification of the β-agonist responses in the heart (5). The fine-tuning regulation of cardiac regulatory protein phosphorylation by protein kinases and phosphatases becomes even more critical in pathological states (29, 53). Heart failure is associated with enhanced noradrenergic activity, which may be compensatory early in the disease state, but long-term neurohormonal activation induces significant damage to cardiomyocytes (53). Increases in type-1 phosphatase (PP1) activity have been observed in end-stage human heart failure, but the role of this enzyme in cardiac function is unknown. Elegant studies by Dr. Kranias’s group have shown that overexpression of the catalytic subunit of PP1 in murine was associated with depressed cardiac function, dilated cardiomyopathy, and premature mortality, consistent with heart failure (11). Ablation of the inhibitor was associated with moderate increases in PP1 activity and impaired β-adrenergic contractile responses (11). Extension of these findings to human heart failure indicated that the increased PP1 activity may be partially due to dephosphorylation/inactivation of its inhibitor. Using adenoviral-mediated expression of a constitutively active I-1 protein (I-1T35D) in myocytes isolated from human failing hearts, we found that we are able decrease PP1 activity and an enhanced contractile function in the presence or absence of β-agonist (11). The design of the I-1T35D construct entailed truncation of the I-1 cDNA to encode for the first 65 amino acids and introduction of nucleotide changes to replace the PKA phosphorylation site (GGT:Thr35) with aspartic acid (GTC:D), resulting in a constitutively active inhibitor. In preliminary experiments, we also found that, in rats with pressure-overload induced heart failure, overexpression of I-1T35D induces an improvement
in contractility. Transgenic expression of a constitutively active inhibitor-1 results in enhancement of phospholamban phosphorylation, augmented cardiac contractility, and attenuation of heart failure development on aortic constriction. Acute adenoviral expression of the active inhibitor-1 halts the progression of cardiac dysfunction and decompensation in a rat model of pressure-overload-induced heart failure. We found that active inhibitor-1 gene transfer results in marked restoration of contractility in rats with pressure-overload-induced heart failure(69). More recently, PKC-α was found to be a critical regulator of contractility through its direct inhibitory effects on I-1 (5, 6). Therefore, multiple lines of evidence are converging toward the critical role that I-1 plays in modulating contractility in heart failure (5).

Another important protein that has been recently targeted is S100A1, which is a member of a multigenic family of low molecular weight Ca2+ cycling proteins and is abundantly expressed in the myocardium with multiple intracellular targets (26). In heart failure, the expression of S100A1 is reduced, and its overexpression by gene transfer induces an increase in SERCA2a and RYR activities, a decrease in Na+, a decrease in NCX, and restoration of creatine phosphate resulting in improved intracellular calcium handling and overall enhanced systolic and diastolic ventricular function in rats following cryo-infarction (60).

The described genetic manipulations of calcium handling and βAR signaling proteins generally result in increased contractility by improved SERCA function. This gain in SERCA function has been reported to result in shortening of cardiomyocyte action potential duration (APD) (77). This physiological effect has the potential to be detrimental due to the inability of contemporary gene transfer technology to achieve complete and homogenous transduction. As a result of this, APD shortening will occur in a heterogeneous pattern throughout the myocardium. This pattern has the theoretical potential to form a substrate for arrhythmias (77).

**Anti-apoptotic strategies**

Myocardial protection by gene transfer encompasses a diverse range of strategies aimed at preventing cardiac cell injury and death. Offending pathophysiologies cause cell death by inflammatory, immunological or oxidant stress-dependent pathways. Cardiac cells respond by activating endogenous protective mechanisms or by undergoing apoptotic and non-apoptotic cell death. Gene transfer approaches have demonstrated a capacity for protecting the myocardium and promoting survival in cardiac cells confronted with the aforementioned insults. Generically, these include anti-inflammatory, immuno-inhibitory, anti-oxidant, and anti-apoptotic or pro-survival approaches.

The transcription factor NF-κB has been the most common molecular target of anti-inflammatory approaches in ischaemia-reperfusion myocardial injury models. The attractiveness of this target lies in the ability of NF-κB to modulate the activity of several downstream genes involved in inflammation. Its role in mediating inflammation in the context of ischemia-reperfusion injury has been demonstrated by blocking its activity using decoy oligonucleotides. In a rat MI model, for example, reduced infarct size was reported after transcoronary delivery of oligonucleotides packaged in HVJ-liposomes. This benefit was conferred irrespective of whether delivery occurred before coronary occlusion or after reperfusion. These positive findings were reproduced in a preclinical porcine model of ischemia-reperfusion injury, following retrograde coronary venous delivery of the above-mentioned oligonucleotides packaged in liposomes. In addition to these, the anti-inflammatory benefits of NF-κB decoy therapy have also been demonstrated in animal models of myocarditis and cardiac allografting.

There is a growing literature on the role of oxidative stress in cardiovascular disease. “Stress” occurs when excess amounts of reactive oxygen species (ROS) are generated and overwhelm the endogenous antioxidant systems resulting in cell death or dysfunction. Gene-based approaches to antioxidant therapy were initially guided by enzyme-based interventions and more recently by the promising findings from experiments involving transgenic mice. In general, an antioxidant effect can be afforded by either reducing ROS production or increasing enzymatic metabolism of these species. Models of the latter approach predominate in the literature. These models usually entail gene transfer of an antioxidant enzyme to the myocardium of an animal subjected to ischemia-reperfusion injury. Myocardial protection after gene transfer has been reported for the enzymes manganese superoxide dismutase and hem oxygenase-1.

Apoptotic myocardial cell death contributes to the pathogenesis of heart failure, irrespective of etiology. As such, molecular targets within the signaling and effector pathways for apoptosis have been pursued in gene therapy models of heart failure in an attempt to attenuate the burden of cell death. Support for this approach includes in vitro data demonstrating that rat cardiomyocytes can be protected from apoptosis-inducing p53 or hypoxia after adenovirus-mediated overexpression of Bcl-2 (anti-apoptotic effector) or PI3-kinase and Akt (pro-survival signaling), respectively. In small animal models of ischemia-reperfusion injury, adenovirus-mediated gene transfer of Akt has been reported to significantly reduce the number of apoptotic cells in the region of injury, limit infarct size, and preserve cardiac hemodynamics. In a different model targeting an apoptotic effector molecule, pacing-induced heart failure was prevented by the intracoronary delivery of adenoviral vector encoding p35, a caspase-3 inhibitor. Undoubtedly, additional anti-apoptotic and cell-survival pathways will be targeted
molecular approaches. A notable recent development is the discovery of an endogenous glycoprotein (gp) 130 receptor-mediated anti-apoptotic pathway. The IL-6 family of cytokines ordinarily signals via this receptor to improve cell survival. Cardiac-restricted ablation of this molecule has been shown to markedly increase the rate of apoptosis after pressure loading the heart. This observation not only highlights the protective role of this signaling pathway against the development of heart failure but also provides a novel molecular target for therapy of heart failure.

**Cardiac conducting system and arrhythmias**

The genetic manipulation of the physiological processes that underlie cardiac excitability and electrical impulse generation and conduction is a small but exciting component within the broader context of gene therapy for cardiovascular disease. Manipulations of this type are achieved by either focal or global myocardial delivery. Examples of physiological effects amenable to focal gene transfer include the creation of biological pacemakers and the modulation of conduction throughout the AV node or reentrant arrhythmia circuits. The threshold for achieving these physiological effects is potentially low due to the requirement for focal genetic modification. In contrast, pathophysiologicals affecting the entire heart, such as genetic forms of the long QT syndrome, impose demands similar to those of myocardial-based pathophysiologicals and are currently limited by inefficient gene transfer.

**Impulse generation and biological pacemakers**

Electronic pacemakers are highly effective treatment for bradyarrhythmias. These devices, however, are prone to lead failure and battery depletion, which necessitate repeat procedures. The burden of repeat procedures is greatest in those who receive pacemakers at a relatively young age. For this reason, the creation of biological pacemakers is conceptually meritorious and deserving of further investigation.

The first approach involved the overexpression of β2AR in cardiomyocytes and right atrium in vivo. The effect of this was to enhance chronotropy. This approach, however, did not confer a capacity for spontaneous diastolic depolarization in gene-modified cells, the hallmark of pacemaker cells. The next approach and the first study to report the generation of a biological pacemaker employed a dominant-negative molecular approach to reduce the current Ik. This current normally suppresses diastolic depolarization by anchoring the cell membrane at a negative resting potential. Adenovirus-mediated transfer of the mutant gene resulted in the generation of dominant escape ventricular rhythms, following transcoronary vector delivery to guinea-pig hearts. Isolation of transduced ventricular cells from these animals revealed a newly acquired capacity for spontaneous diastolic depolarization. More recently, biological pacemakers have been created by the focal transfer of the HCN gene that encodes for the ion channel mediating the pacemaker current, If (67a). In a canine model, adenovirus-mediated HCN gene transfer to the left atrium was reported to result in shifting of the escape pacemaker from its normal position in right to the left atrium following recovery form vagally induced asystole. These studies provide proof-of-principle support for the creation of biological pacemakers. These pacemakers, however, do not sufficiently approximate the physiology of the sinoatrial node, and hence this technology requires further refinement. More recently, a bioartificial sinus node constructed via in vivo gene transfer of an engineered pacemaker HCN channel reduces the dependence on electronic pacemaker in a sick-sinus syndrome model (78). These results provided the basis for HCN gene-based therapy for correcting defects in cardiac impulse generation.

**Modulating impulse conduction**

Cardiac impulse conduction can be modulated by either altering excitability or intercellular coupling. To date, genetic manipulations have targeted the former electrophysiological property to affect electrical conduction. The only published in vivo model reported slowing of AV nodal conduction following focal gene transfer and overexpression of the inhibitory G-protein (Gi2) (23). In this porcine model, adenoviral vector was delivered to the AV node by selective catheterization of the AV nodal artery. The biological effect of Gi2 overexpression is to emulate β2AR blockade. As a result, gene transfer induced prolongation of PR and AH intervals and also reduced the ventricular response rate to adrenergic stimulation and induced atrial fibrillation. Additional approaches to slowing conduction are preliminary and include the in vitro data from cocultures of cardiomycocytes and fibroblasts that have been genetically engineered to express a repolarizing current. This study reported slowing of electrical propagation in cardiomycocytes cultured with these gene-modified fibroblasts.

**Targeting repolarization**

Abnormalities of repolarization resulting from inherited or acquired ion channel defects can give rise to potentially fatal ventricular arrhythmias (78). Advances in the understanding of the molecular basis of these arrhythmic syndromes have increased the prospect of gene-based treatments. In vitro studies have confirmed the capacity for this technology to correct the cellular electrophysiological abnormality of prolonged APD that is common to many of these syndromes. Extending these findings, in vivo gene transfer has also been reported to result in potentially important physiological effects such as shortening of the QT interval in genetically normal guinea pig
hearts. More recently, phenotypic correction has been reported in a transgenic mouse model of long QT syndrome after gene transfer restored the dysfunctional ion channel.

Conclusion and Future Prospects

The clinical cardiovascular gene therapy experience is very limited. The available data, however, emphasizes the importance of randomized placebo-controlled trials in assessing the efficacy of novel treatments. Larger trials of this type are in progress, and the results of these are eagerly awaited. Meanwhile, the large volume of preclinical data is likely to continue to expand with increases in knowledge of the molecular basis of cardiovascular disease. Importantly, the accumulation of this data provides the scientific basis for which to pursue the human evaluation of novel gene-based therapies.

With regard to gene transfer technology, recent serious adverse reactions in young human subjects have been attributed to the vector employed. As a result, efforts to improve vector biosafety have been appropriately revitalized. With these efforts, vectors will hopefully undergo modifications to further improve biosafety, in addition to gene transfer efficiency. Conventional treatment modalities have made significant advances over the last decade in the areas of pharmacotherapy, vascular intervention, surgery, and implantable devices. The modest success of gene therapy in cardiovascular disease thus far has been in filling a therapeutic need lacking effective therapy. Future success will depend on either exceeding conventional therapies or finding effective gene-based therapies. In the next few years, a number of phase 1 clinical trials using AAV based gene therapy (as mentioned earlier) targeting patients with heart failure will hopefully provide much needed new information in this field.

This study was supported in part by National Heart, Lung, and Blood Institute Grants RO1 HL-078691, HL-057263, HL-071763, HL-080498, and HL-083156, and a Leducq Transatlantic Network (R. J. Hajjar)

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