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Surgical methods for cardiac gene transfer

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“Scientific truth passes through three stages. First, it is ridiculed. Second, it is violently opposed. Third, it is accepted as being self-evident.”

– Arthur Schopenhauer [1].

Over the last few decades, progress in the molecular biology of cardiomyocytes and the decoding of their genome through DNA sequencing has improved our insight into the pathophysiological mechanisms underlying the development of many heart diseases. The further evolution of genetic methods to investigate signal transduction, microarray analysis, and the development of transgenic animal models of various diseases including myocardial hypertrophy and others has contributed to the emergence of cardiovascular gene therapy as an important new paradigm in clinical and experimental research. These factors have resulted in the translation of cardiovascular therapy from animal studies to clinical applications more rapidly than expected.

Successful plasmid DNA transfection after direct injection into beating rat hearts was demonstrated by expression of a recombinant β -galactosidase gene after 3–4 weeks. These results showed for the first time that cardiac muscle has a unique ability to take up and express injected recombinant DNA without recipient cell division [2]. The next historical step was the proof that viral-mediated gene transfer after intramyocardial injection is thousands of times more efficient than plasmid DNA; however, the percentage of cardiomyocytes expressing marker genes was largely limited to regions adjacent to the injection site [3,4]. Subsequent development involved intracoronary infusion of adenovirus. Viral DNA was detected in the rabbit myocardium for 2 weeks after delivery, although significant gene expression in other main organs was also recorded [5]. These and other data obtained two decades ago showed that: the route of gene delivery is no less important than the choice of vector system; and the basic methods of cardiac gene transfer were primarily

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intramyocardial injection and intracoronary infusion. Over time, although, it became clear that both of these methods had severe limitations and did not adequately reflect all the potentially more novel technologies of gene delivery. A classification of existing cardiac gene delivery techniques was proposed that considers variations of heart perfusion during transfer, site and method of injection, and interventional approach [6]. These various gene delivery strategies each have their own barriers that must be overcome. For example, vector-mediated transvascular gene transfer has to overcome blood cells, the capillary layer, the vascular endothelial lining, the extracellular matrix, the cell membrane and the nucleus.

Direct myocardial delivery

Methods for direct gene delivery include intrapericardial and myocardial routes. The latter approach uses either an epicardial or endocardial approach. Since the pericardium is not only the cavity surrounding the heart but also a squamous serous membrane secreting vasoactive substances, it was speculated that virus injected intrapericardially might diffuse through this barrier to the underlying myocardium and coronary arteries [7]. Initially, encouraging expression in the parietal pericardium was found, but it was then demonstrated that the amount of vector has to be unreasonably vast in order to penetrate the myocardial wall [8]. Thus, the intrapericardial route is not likely to be particularly promising in clinical applications. Direct intramyocardial techniques are very attractive and currently being used due to the fact that they are simple and reproducible, allow for targeting of specific areas of interest and achieve high local vector concentration. Application of this method has resulted in successful therapeutic angiogenesis [9], and focal arrhythmias therapy [10]. However a key limitation of this method includes the fact that transgene expression is limited to the injection sites so the method is therefore not sufficient to achieve a global distribution. Needle injection also causes a secondary acute inflammatory and innate immune response because of mechanical cardiomyocyte injury with the subsequent appearance of fibrous tissue. Moreover, this route of transfer does not preclude systemic vector spread as was hoped. Relatively low transfection efficiencies and other disadvantages of needle injection led to the use of a variety of physical and mechanical methods in addition to intramyocardial gene transfer. These are widely used, particularly with nonviral vector injection. The most well-known and effective is electroporation, which involves the application of short duration, high intensity electric pulses that amplify cardiomyocyte membrane permeability and increase DNA uptake. Electroporation can increase gene expression by 100- to 1000-fold. Sonoporation is another commonly used method involving the attachment of plasmid DNA to gas-filled micro-bubbles that are then destroyed by ultrasonic pulses. Other less common methods include laser irradiation and magnetic field-based transfection. A laser's thermal effect can produce tiny holes in cell membranes and enhance permeability to exogenous DNA. Magnetofection employs the principle of transferring paramagnetic nanoparticles containing a vector via the influence of a strong magnetic field. Given the presence of islet-like vector clusters around the site of injection with surrounding accumulation of host inflammatory cells after gene delivery via needle, some authors believe that liquid jet injections and gene gun particle bombardment help in addressing these issues. The epicardium, which consists of loose connective tissue and fat, presents a relatively more difficult to bypass barrier to the myocardium, which is composed almost completely of

cardiomyocytes, than the endocardium, which is a smooth membrane of endothelial cells. Comparative evaluation of several approaches indicated that endomyocardial injection was associated with 43% microsphere retention and transepical access with only 15% [11]. However an endocardial approach is possible only through cardiac catheterization using an image guidance system. The most popular device for image-guided endocardial injection is the NOGA system, which utilizes electromagnetic field sensors to analyze information from intracardiac ECGs. Alternatives to NOGA include cardiac imaging modalities with echocardiography, MRI and x-ray fluoroscopy.

Transvascular gene delivery

Selective coronary catheterization with antegrade delivery was first tested in a rabbit model [5]. Today, it is the technique utilized in a clinical trial of heart failure gene therapy. The benefits of this method include the possibility of repeated vector deliveries to the whole myocardium with homogenous gene expression and minimal invasiveness through well-developed percutaneous coronary procedures. However, the limited transduction and varied results with systemic leakage leading to significant collateral organ uptake led researchers to identify parameters influencing the efficiency of intracoronary transfer. These parameters include the contact time of vector in coronary circulation, intravascular flow rate and perfusion pressure, composition of perfusate and endothelial permeability [12,13]. There have been many new techniques to complement antegrade delivery: brief interruption of coronary flow [13], concomitant coronary venous blockade [14], increased perfusion pressure and flow [15], and transient cardiac arrest and enhanced endothelial permeability with pharmacological agents [16,17]. The next technological advancement was selective retrograde delivery through the coronary sinus. Pressure regulated retrograde infusion into the anterior cardiac vein substantially increases gene expression in the targeted territory due to increased coronary passage time of more than tenfold compared with other methods [18]. Another advantage is an increased capillary filtration ratio in the venous part of the capillary bed and the ability to overcome the resistance of precapillary sphincters located before arterial capillaries [19]. However, one of the most serious shortcomings of single pass transvascular gene transfer is the very fast dilution of vector concentration in the circulating blood with subsequent gene and vector dissemination to collateral organs. This limitation encouraged researchers to develop closed-loop recirculatory systems, which allowed separation of the coronary vascular bed from the systemic. The first design and use of such a system was by Bridges *et al.* [20]. The principal strength of this technology includes a dramatic (>100-fold) increase in transduction efficiency, the extension of vector residence time, the ability to manipulate endothelial permeability, the avoidance of an immune response to the vector and the ability to washout the vector post gene delivery limiting collateral organ exposure [21]. It is estimated that about 30% of patients with coronary artery disease cannot undergo revascularization procedures. Under these circumstances the use of transvascular intracoronary wall delivery with gene-eluting stents seems very attractive [22]. The advantages of these devices include extensive clinical experience in coronary catheterization interventions, safety, existence of permanent scaffold structure, and the fact that stents are a good container allowing gradual and local gene release over time without side effects.

Future perspective

Randomized clinical trials have suggested that the therapeutic benefits of gene therapy are not as substantial as expected from animal studies. This discordance in results is largely due to gene delivery methods that may be effective in small animals but are not scalable to larger species and, therefore, cannot transduce a sufficient fraction of myocytes to establish long-term clinical efficacy. Ideally, an optimized gene transfer should incorporate: retrograde route of delivery through the coronary venous system; vector washout after transfer; increased myocardial transcapillary gradient for viral particles; a closed-loop for extended transgene residence time; and myocardial ischemic preconditioning.

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Biographies

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