REVIEW

Contemporary biological therapies for cardiovascular diseases

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Abstract: Cardiovascular diseases are top cause of mortality in the world. Current interventional therapy and pharmacotherapy may alleviate symptoms or slow disease progression but are unable to cure or treat them. Molecular and pathophysiological advances have paved the way for contemporary biological therapies to be tested and standardized for the treatment of these diseases. Stem cells therapy and gene therapy has shown promise in the treatment of CVDs. Various types of stem cells used in cardiac conditions like myocardial infarction with the aim of regenerating the damaged myocardium have had variable success rates in clinical and preclinical trials. Improvements in methods and routes of cell delivery have improved clinical outcomes. Gene therapy employs therapeutic genes to treat diseases. Advances in vectors have improved transfection efficiencies and transgene expression and enhanced role in Heart failure, ischemic disease as well as arrhythmias. Clinical trials have shown improved cardiac function upon treatment with genes which promote angiogenesis. The current review looks at the role of these biological therapies in cardiovascular diseases.

Keywords: Cardiovascular diseases, stem-cell therapy, gene therapy, biological therapy

INTRODUCTION

According to the WHO, the number one cause of mortality in the world is cardiovascular disease. The prevalence of CVD is constantly increasing, clinical outcome with conventional therapy remains poor and the resultant economic burden makes it a critical public health issue. According to an estimate, cardiovascular diseases accounted for 17.5 million (31%) of deaths worldwide. Each year, heart disease kills over 600,000 people in the United States. In 2013, 1 in nine deaths were attributed to heart failure, while 1 in seven deaths were a result of coronary artery disease (Bleumink *et al.*, 2004; Kochanek *et al*., 2014; Mozaffarian *et al*., 2016; "WHO Cardiovascular diseases (CVDs)," 2016). Although current therapeutic approaches towards CVD and HF alleviate symptoms and retard the progression of disease, they do not cure the disease. Advances in the understanding of the pathophysiology and elucidation of molecular pathways for these diseases have paved the way for alternative, more efficient, therapeutic strategies to treat and possibly cure cardiovascular diseases. Thus it has become viable to use biological therapies such as gene (Tilemann *et al.*, 2012) and cell therapy as novel approaches to treat cardiovascular abnormalities (Segers and Lee, 2008).

Stem-cell therapy

Stem cells are self-renewable, undifferentiated cells, having the ability to divide into progenitor cells, that can differentiate into various lineage specific mature cells (Segers and Lee, 2008). The inevitable deterioration of cardiac function in various cardiac diseases cannot be circumvented by current therapeutic approaches. Thus, interest in novel therapies, aimed at regeneration of the myocardium have increased. Cardiac regeneration can be obtained by two methods: Stem cells can be implanted directly into the myocardium at the injured site; vascular regeneration and cardiomyocyte replacement can be obtained by induction of endogenous repair mechanisms (Laflamme and Murry, 2011). Myocardial damage, as a result of ischemia and infarction, decreases the pumping capacity of the heart and makes it susceptible to life-threatening arrhythmias. Endogenous cardiac repair mechanisms like resident stem cell recruitment and maturation exist but are insufficient for the replacement of extensive cardiomyocyte loss (Gonzalez *et al.*, 2008). Thus, the ability of the heart to naturally regenerate is not enough to prevent heart failure (Lyngbæk *et al.*, 2007). This justifies the use of exogenous stem cells for cardiac regeneration to better cardiac function.

Types of stem cells used in cardiac repair

Based on their differentiation ability, stem cells can be classified into totipotent stem cells, pluripotent stem cells and multipotent stem cells. Totipotent stem cells are the cells which have the ability to generate a complete organism, given that they are obtained from the uterus of an animal. Pluripotent stem cells have the potential to divide into all types of cells, with the exception of extra-embryonic tissue cells, similar to those found in the placenta. Induced pluripotent stem (iPS) cells and embryonic stem (ES) cells are both pluripotent in nature and thus cannot divide into a complete organism. Multipotent stem cells are able to divide into a few limited lineage types (Sun, 2009).

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Pluripotent stem cells

The blastocyst stage of the embryo is a popular source of embryonic stem cells (ESCs). These cells are extracted from the inner cell mass, and are termed as human embryonic stem cells (hESC) when derived from human embryo. They have a pluripotent nature which means they have the potential to differentiate into the cells of the three germ layers. They can also be propagated in culture medium, which means they can be induced to differentiate into cardiomyocytes under appropriate conditions. The specific conditions, signaling pathways as well as transcription factors need to be investigated, so as to direct the differentiation in the desired direction. Because, in theory, the hESCs can generate cells of the mesoderm, ectoderm as well as the endoderm (Habib *et al.,* 2008; Lev *et al.,* 2005)

The clinical application of hESC is limited, due a number of reasons. Firstly, ethical issues arise, since obtaining hESCs involves the killing of the human embryo at an early stage. Secondly, these cells show low efficiency, since grafted cells produces only small cardiomyocyte populations in both mice and humans. Furthermore, their pluripotent nature infers them the ability of forming teratomas. Finally, hESC therapy has an associated risk of immune reaction (Amit *et al.,* 2000; Lo and Parham, 2009; Nussbaum *et al.,* 2007). Induced pluripotent stem cells (hiPSCs), on the other hand, do not face any ethical issues, thus do not have these limitations (Manuscript and Proximity, 2011).

Skeletal myoblasts

Skeletal myoblasts are progenitor cells found in skeletal muscles, which act to repair injured muscle tissue. As shown by several animal studies, myoblasts when injected into the infracted myocardium, improve the ejection fraction and decrease remodeling. Although, only a small number of these cells survive upon injection, they differentiate, after proliferation, into multinucleated skeletal myotubes. The myotubes do not form gap-junction proteins nor do they connect with cardiomyocytes. However, they do align with the host cardiomyocyte to serve their function. They are unable to differentiate into cardiomyocytes, which serves as an advantage, in the sense that teratoma formation is not an issue with these cells (Menasché, 2008, 2007).

Bone marrow derived stem cells

Hemangioblasts and Mesenchymal stem cells (MSCs) form 0.01% of the human bone marrow. Red blood cells, lymphocytes, endothelial progenitor cells, megakaryocytes and myeloid cells are products of hemangioblasts. Whereas, myocytes, osteoblasts, adipose cells and chondrocytes are derivatives of MSCs. It was perceived that MSCs and hemangioblasts have the capability of trans-differentiation into cardiomyocytes and endothelial cells upon implantation into the damaged myocardium (Gnecchi *et al.,* 2005; Kinnaird *et al.,* 2004). MSCs are pluripotent progenitor cells with no surface markers (antigens) and are thus considered immunologically privileged. In addition, secretion of paracrine factors by MSCs, for the regulation of immune system and mediation of inflammatory responses makes MSCs an attractive choice for regenerative purposes. (Krause *et al.,* 2010; Pittenger, 1999) Due to these qualities, allogenic grafting of MSCs can be done, circumventing the need for immunotherapy.

MSCs have been reported to generate cardiomyocyte through myeloid intermediates. Using myeloid derivatives may prove to be effective for cardiac therapy (Fukata *et al.,* 2013). Cardiovascular repair using hematopoietic stem cells (HSCs) is advantageous since transplantation results in angiogenesis and myogenesis, which is the basic requirement of cardiovascular repair. Nevertheless, elucidation of signaling pathways that regulate how HSCs proliferate and differentiate is necessary for optimum therapy. In addition, tumorigenesis is also a concern with these cells (Asahara *et al.,* 2000). Specific induction of HSCs into cardiomyocytes must be guaranteed to avoid tumorigenesis.

Cardiac progenitor cells

It was thought that the heart was a terminally differentiated organ, without an ability to regenerate and repair. However, Bergmann *et al*. revealed 1% cardiomyocyte renewal each year, in young adults (Bergmann *et al.,* 2009). Theoretically, cardiac stem cells are strong candidates for use in cardiac regeneration. Due to the fact that they have the ability to generate cardiomyocytes as well as surrounding tissue resulting in better contractility and increased vascularization (Beltrami *et al.,* 2003).

Warton's Jelly derived mesenchymal stem cells

Direct injection of Warton's Jelly derived mesenchymal cells into the infarcted myocardium showed promising results. The cells survive and differentiate into endothelial cells as well as cardiomyocytes. In porcine model with acute myocardial infarction, WJ-MSCs promoted cardiac stem cell recruitment as well as differentiation and improved ventricular remodeling due to enhanced viable myocardium and decreased apoptosis and fibrosis (Zhang *et al.,* 2013).

Methods and routes of cell delivery

There are three major routes of stem cell delivery: Intravenous (IV), Intracoronary (IC) and intramyocardial (IM) (Dib *et al*., 2010). The route of cell delivery most frequently used in clinical practice is the IC method. The route is preferred because, it leads the cells directly to the affected site via the coronary vasculature and is most frequently employed during percutaneous coronary interventions after acute MI (Dib *et al.,* 2011). There are two ways of infusion in the catheter, infusion of cells at variable rate without interruption of coronary blood flow,

or interruption using a balloon, also known as the stop-flow method. IC delivery requires cellular migration to the target, which is partly directed by stem cell homing. Stem cell homing is a phenomenon in which cells migrate to their target organ, which in this case is the damaged myocardium. They are mostly homogenously distributed and are retained by adhesion to the endothelial layer of the coronary vasculature (Dib *et al.,* 2010).

Intramyocardial injection is an invasive procedure which involves direct injection of stem cells to the myocardium. Injections can be made, during surgery via the epimyocardial route or with a needle tipped delivery catheter, transendocardially. Epicardial injections are considered safe, with high myocardial cell retention. However, there is a chance of ventricular perforation and systemic embolism. There is a heightened risk of inflammation associated cardiac arrhythmias. The transendocardial approach employs a NOGA guided percutaneous catheter to deliver the cells to the damaged myocardium. It is a relatively safer approach with retention efficiency greater than IV infusion. But has a similar risk profile as epicardial injection (Dib *et al.,* 2011).

Intravenous infusion of stem cells is a low risk and easy method of administration. However, the success of this method depends largely on cell homing. Preclinical data revealed that the absolute level of intravenously injected EPCs homing to the heart is 1% of the injected cells. A significant amount of cells are entrapped by the lungs (Barbash *et al.,* 2003).

Gene therapy

Gene therapy is defined as an experimental technique for the treatment or prevention of particular disease states, using defined genetic material. It is an alternative to drugs and surgery and requires genes to be inserted into specific target cells. The three basic components of gene therapy are: Identification and isolation of the mutated gene; delivery of healthy gene to patient using appropriate vectors; regulation of gene expression. Gene therapy encompasses replacement of mutated genes, alteration of mutated gene or addition of a new gene (Jain, 2011; McCain, 2005). After the identification of affected gene, a clone of the healthy gene is made. The appropriate vector is loaded with this therapeutic gene and delivered to the target cells. By employing an appropriate delivery system, the gene is delivered to the nucleus of the target cell.

Vectors

Non-viral vectors

The simplest method of gene delivery is the direct injection of naked plasmid DNA (pDNA), which is a safe and inexpensive method. However, its inefficient transfection of host cells has led to advancement in non-viral vector system of gene delivery, which now employs synthetic and natural compounds complexed with DNA to transfer genes from the site of injection to the target site. These vectors include synthetic peptides, cationic polymers and cationic liposomes. Complexes of cationic liposome and DNA are called lipoplexes and are one of the most efficient non-viral gene delivery system. They provide a cheap method of carrying large molecules to target site. They may also be targeted to specific tissues whilst protecting DNA from nucleases. Polymer based vectors employ several kinds of lipids to achieve effects similar to lipoplexes (Kamimura *et al.,* 2011; Ramamoorth and Narvekar, 2015). In-vivo use of these vectors has been found to form aggregates and accumulate in various tissues.

Physical methods are used to increase efficiency of specific tissue delivery as well as increase cell membrane permeability of therapeutic agent. Physical methods employ hydrodynamic force, electric shock or mechanical pressure to achieve these effects. Physical methods, with a potential of cardiomyocyte transfection include electroporation (Mali *et al.,* 2008) and ultrasound targeted micro bubble (UTM) (Ramamoorth and Narvekar, 2015). UTM has gained significant traction for treatment of CVD because of its low immunogenicity and toxicity coupled with re-administration potential and targeted gene delivery (Ferrara *et al.,* 2007). UTM delivery of DNA have proven to be advantageous in HF and MI animal models. Ventricular function and myocardial perfusion were seen to improve in a rodent model, treated with VEGF loaded lipid micro bubbles (Fujii *et al.,* 2009).

Non-viral gene therapy is bio-safe, inexpensive, easily produced and has low pathogenicity. However, low transgene expression due to low efficiency of gene delivery has decreased its applications (Glover *et al.,* 2005).

Viral vectors

Viruses containing genetic material bind to cell surface receptors. They are internalized and delivered to the target cells. The viral protein coat and lipid envelope direct the therapeutic gene towards the target, protecting it from lysosomal degradation (Kay, 2011; Petrus *et al.,* 2010). Thus viral vectors are generally more efficient than non-viral vectors, however immunogenicity and cytotoxicity caused by viral vectors limits clinical use (Mingozzi and High, 2013).

Adenoviral vectors

Commonly use viral vectors include adenoviral vectors, adeno-associated viral vectors and lentiviral vectors. Adenoviral vectors efficiently transduce dividing as well as non-dividing cardiomyocytes. They enter cells by binding with the coxsackie-adenovirus receptor (CAR) and are endocytosed (Parker *et al.,* 2008; Wasala *et al.,* 2011). This vector system is useful for angiogenic therapies in peripheral arterial occlusive disease (Muona *et al.,* 2012) and ischemic heart disease (Stewart *et al.,*

2006). Transduction efficiency is particularly high in cardiomyocytes due to high expression of CAR. In addition, intracoronary infusion and direct intramyocardial injection yield high levels of transduced cardiomyocytes (*Saaristo et al.,* 2005; Williams *et al.,* 2010).

Adeno-associated viral vectors

The single stranded (ss) DNA vectors have the ability to persistently produce transgene expression in the heart. Compared to Ad vectors, they have a much better safety profile and produce lesser inflammation. Among many serotypes AAV1, AAV6, AAV8, and AAV9 are considered to be the most cardiotropic (Mingozzi and High, 2011; Rabinowitz, 2014; Vandendriessche *et al.,* 2007).

Lentiviral vectors

Derived from HIV type 1, lentivirus vectors are single stranded (ss) RNA, enveloped vectors. Since they can integrate their genome into both dividing and non-dividing cells, as cDNA, they are candidates for producing long-lived therapeutic gene expression. Their poor transduction of the myocardium limits their use in CVD/HF. However, monogenetic hematopoietic disorders have been treated successfully with LV. Therapeutic angiogenesis can be induced using Lentiviral gene therapy by transduction of endothelial progenitor cells and endothelial cells.

Enhancing uptake of viral vectors

For the improvement in gene delivery and vector uptake by cardiomyocytes, various methods have been developed. Vector delivery methods improved from percutaneous intracoronary viral delivery to NOGAw based direct intramyocardial injections (Gyöngyösi *et al.*, 2005). However these advances were not contributing enough to the transduction efficiencies. Higher transduction efficiencies required higher doses, which increased the chances of ectopic transduction in other tissues. An 80% increase in cardiac transduction was observed when VEGF was combined with calcium, nitroglycerine and adenosine infusion before the administration of vector. This lead to hemodynamic instability.

Applications in cardiology

The third most popular application of gene therapy is, cardiovascular disease. It is an alternate to conventional pharmacotherapy and is thought to be advantageous for refractory conditions. Cardiovascular gene therapy is known to be effective for therapeutic angiogenesis, prevention of bypass graft failure, reduction of apoptosis, and myocardial protection. These effects are produced due to gene therapy with certain growth factors like VEGF, HGF and FGF. Gene therapy has also been found to be beneficial for amplification of myogenesis in stem cell therapy. Cardio-protective effects have been demonstrated in animals treated with antioxidant coding genes. These proteins code for anti-apoptotic proteins like mitogen activated protein kinase, eNOS, HSP among others.

Clinical trials have been conducted for ischemic diseases like myocardial ischemia and CAD. Several trials have demonstrated the effectiveness of gene therapy indicated by improved cardiac function. Treatment of heart failure using gene therapy has been explored by several clinical and preclinical trials. Therapeutic genes used for HF involve SERCA2a, SDF-1 and AC6. It was found that there is a decreased expression and function of SERCA2a in heart failure. However improved function was observed upon treatment with SERCA2a gene. Several preclinical studies are underway to determine and evaluate various targets for gene therapy of arrhythmias. Recent research is directed towards improvement of gene transfection techniques and gene expression regulation for the improvement of clinical outcomes (Ginn *et al.,* 2013; Lavu *et al.,* 2011; Wolfram and Donahue, 2013).

CONCLUSION

Advances in gene and cell therapy have significantly impacted how we look at cardiovascular treatment. Present research in biological therapies has made it evident that it is possible to not only treat but cure cardiovascular diseases. Use of exogenous stem cells for cardiac tissue regeneration is certainly effective and the future of stem cell therapy looks bright.

Furthermore, progress in gene therapy has made it possible to treat cardiovascular diseases. Gene therapy not only has the potential to increase angiogenesis and decrease myocardial cell death, buy can also be used to augment the efficiency of stem cell therapy. However the choice of transfection method has become increasingly complex. Although UTM has a low toxicity potential and can be used repeatedly for targeted therapy, Low transgene expression efficiency has limited the use of non-viral vectors for gene therapy. Conversely, viral vectors have limited use due to their cytotoxic potential, even though they are more efficient.

As molecular research sheds light on the signaling pathways, transcription factors and tissue differentiation, the potential for regenerative therapy increases. However, challenges such as tumorigenesis, teratogenesis and cytotoxicity associated with these method currently limit their use. Further research is necessary to completely implement these methods of myocardial repair in practice settings.

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