International Journal of Advanced Research and Review

www.ijarr.in

GENE THERAPY FOR CARDIOVASCULAR DISEASES

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ABSTRACT

Gene therapy is the insertion of genes into an individual's cells and tissues to treat disease using experimental techniques. This was done by replacing a mutated gene that causes disease with a healthy copy of the gene and when in activating a mutated gene that is functioning improperly(Müller, Katus and Bekeredjian, 2017). Gene therapy in cardiovascular disease must be aimed at correcting key molecular mechanism in cardiac tissue. This requires introduction of DNA/RNA that targets specific cardiomyocyte processes that alter the cardiovascular disease outcomes(Lowenstein *et al.*, 2000).

One of the key proteins defective in heart is SERCA2a. SERCA2a expression and functions are decreased in cardiovascular diseases. This decease reduces calcium transient that is characteristic of systolic heart disease. The Calcium Upregulation by Percutaneous administration of gene therapy in Cardiac Disease (CUPID) trial looked at the safety and efficacy of SERCA2a in cardiac gene therapy(Murphy *et al.*, 2010).

In cardiovascular gene therapy, viral vectors genetically modified retroviruses, lentiviruses, adenoviruses, adeno-associated viruses are used. Viral vectors are made replication deficient to ensure safety, but require large amounts of vector particle for efficacy (Williams *et al.*, 2010).

Key words: Cardiovascular, Gene therapy, Calcium handling, Vectors

INTRODUCTION

Gene therapy is designed to introduce genetic material into cells to compensate for abnormal genes or to make a beneficial protein. In accordance with that using gene therapy technique originally developed to deliver DNA or RNA molecules as genetic materials to cells/tissues for the treatment of genetic diseases. Mainly gene therapy is used for replacement of ineffective genes for the treatment of hereditary diseases and enhancement of the normal gene activity or introduction of additional gene information affecting the course of diseases associated with cell proliferation (Rissanen and Ylä-herttuala, 2007).Gene therapy and tissue engineering have also converged for the repair of various tissues/organs, such as musculoskeletal and cardiovascular systems (Tilemann and Hajjar, 2011).

Cardiovascular diseases such as coronary artery diseases (CAD), peripheral vascular diseases, vein graft failure, ischemic heart diseases (IHD) and heart failure (HF) remains the leading causes of morbidity and mortality in developed countries. These cardiovascular diseases are characteristically localized, site-specific targeting of gene therapy for the cardiovascular system (CVS) (Katz *et al.*, 2012).Cardiovascular gene therapy should ideally deliver the

genetic material to a specific target and reaches a level of expression sufficient for therapeutic actions and this approaches shifts the therapeutic focus towards correcting the pathophysiology at the cellular and subcellular level (Hulot, 2017).

In cardiovascular gene therapy, cardiac gene delivery is important to be accompanied by a need to deliver the therapeutic genes to diverse vascular cell types including vascular smooth muscles (SMC), endothelium, myocardium, or tissues that influence lipid metabolism. The gene delivery methods have been developed using both viral and non-viral based on their vectors. This vector systems and gene delivery technologies must, therefore, be developed for individual applications (Nabel, 1995;Isner, 2002).

A basic premise of cardiac gene therapy is the availability of vehicles that can robustly, specifically and persistently deliver therapeutic genetic materials to the heart without generating local and/or systemic toxicity. In cardiovascular gene therapy broadly explain the gene delivery vectors can be divided into two categories: non-viral and viral vectors (Nabel, 1995).

Cardiac gene delivery system

In cardiovascular gene therapy, there are four methods, which were developed by using both, viral and non-viral based on their vectors. They are a cell-based genetic modification, ex vivo gene therapy, local gene delivery in vivo and systemic gene delivery (Lowenstein *et al.*, 2000).

1. **A cell-based genetic modification**

The ex vivo transduction to express therapeutic genes used to treat cardiovascular diseases. This gene delivery system is not like other inherited genetic defects which may require more long-term gene transfer, transient, non-integrative gene expression (Hulot, Ishikawa and Hajjar, 2017).

2. **Ex vivo gene therapy**

In ex vivo, genetic modification of cardiovascular tissue is preferred as it allows the cardiac delivery of therapeutic genes to the target tissue in different ways. This method is mainly used for gene therapy of vein graft failure.

3. **Local gene delivery in vivo**

Under local gene delivery system, the ischaemic myocardium or atherosclerotic coronary arteries diseases target vascular tissues are inaccessibility. In cardiovascular gene therapy, there are different techniques used to date for in vivo cardiac gene transfer. They are,

- Coronary perfusion
- Intra-myocardial injection
- Pericardial injection
- Aortic clamping
- Cross-clamping of the aorta and pulmonary artery(Hajjar *et al.*, 2000)

3.1 Coronary perfusion

Coronary perfusion commonly occurs during heart relaxation (diastole) when the subendocardial coronary vessels are open and under lower pressure. In this coronary perfusion, the flow never comes to zero in the right coronary artery, since the right ventricular pressure is less than the diastolic pressure (Collins and Thrasher, 2015). This gene

transfer method is done by using coronary perfusion method in cardiovascular gene therapy (Fishbein, Chorny and Levy, 2011).

3.2 Intra-myocardial injection

In this in vivo method the targeting genes to the heart though transfer allows and assess the efficacy of highly specific intervention in heart (Rincon, VandenDriessche and Chuah, 2015). This method is cause additional death of cardiomyocytes in ischaemic heart disease(Venugopal *et al.*, 2012).

3.3 Pericardial injection

The major aim of doing this is specialist in treating this condition are reduction of the inflammation of the pericardium and the pain, finding and ruling out further complications.

3.4 Aortic clamping

The aorta may be clamped at many sites along its course from the upper chest down to the level of the navel. This is not done at the root of the aorta but elsewhere along its course. This allows blood to be diverted safely to other parts of the body(Isner, 2002).

3.5 Cross-clamping of the aorta and pulmonary artery

This is used to isolate the heart from the rest of the body's circulation during procedures on Cross-clamping of the aorta and around the heart (Wolfram and Donahue, 2013).

4. Systemic gene delivery

Systemic gene delivery system occurs under the in vivo administration of viral vector system. Due to the efficient uptake of systemically administered vectors the liver, cardiovascular gene therapy are limited this delivery system because of in vector technology. This systemic gene therapy has been used for the treatment of hypercholesterolaemia and hypertension (Lowenstein *et al.*, 2000).

Mainly, there are two types of gene-targeted methods used in cardiovascular gene therapy. They are transductional targeting and transcriptional targeting method. Transductional targeting system alters the natural infection pathway and can be performed by pseudotyping, whereby modulation of the viral envelope allows manipulation of viral vector cell targeting specificity. Transcriptional targeting via tissue-specific promoters has also been investigated. A suitable cardiac-specific promoter is the myosin light chain. An additional benefit of the tissue-specific promoter is the reduction of gene expression in antigen presenting cells, thus reducing the host immune response (Jones and Koch, 2005).

Calcium handling in heart

In the heart, Ca^{2+} is tightly regulated at several levels. When the movement of calcium, the sarcoplasmic reticulum (SR) plays an important role in each contraction and relaxation. Cardiomyocyte depolarization induces an influx of Ca^{2+} from the extracellular milieu via the voltage to depend on L- type Ca^{2+} channels and allowing entry of the small amount of Ca^{2+} into the cell (Isner, 2002).Through the coupling of the L-type Ca^{2+} channels and this, in turn, activates the ryanodine receptor (RyR), the SR release channels to release the larger amount of intracellular stores of Ca^{2+} to activate the myofilaments and leading to contraction. During relaxation, Ca^{2+} is reaccumulated in the SR by the SR Ca^{2+} ATPase pump (SERCA2a) reduced expression or activity of the SR and leads to increased concentrations of cytosolic Ca^{2+} and less available Ca^{2+} for release from the SR (Seong and Bae, 2009).

In this time Ca^{2+} exchange extracellularly by the sarcolemmal Na/Ca exchanger. The predominant isoform in cardiomyocytes (SERCA2a) activity is influenced by phospholamban. This helps Ca^{2+} pumping inhibition of PLN is another approach to improve Ca^{2+} handling. This Ca^{2+} handling causes an important change in the ratio to PLB to SERCA2a, with its relative increase in the failing heart and the inhibitory effect of PLB being even greater (Hajjar *et al.*, 2000). In the unphosphorylated state, phospholamban inhibits the Ca^{2+} ATPase, whereas phosphorylation of phospholamban by cAMP-dependent protein kinase and by Ca^{2+}calmodulin-dependent protein kinase reverse this inhibition. Moreover in the unphosphorylated state PLB inhibits the Ca^{2+} ATPase expression decreased diastolic Ca^{2+} which is a result that might be expected to prevent activation of signaling molecules including calcineurin whereas phosphorylation of phospholamban by cAMP-dependent protein kinase and by Ca^{2+}calmodulin-dependent protein kinase reverse this inhibition. This is capable of including myocyte hypertrophy and cell death (Greenberg, 2015).

In cardiac muscle strips, the Ca^{2+} pump SERCA2a causes muscle relaxation by lowering the cytosolic calcium and restores the calcium reserves in the SR. Then SR is decreased Ca^{2+} ATPase activity and Ca^{2+} uptake appears responsible for abnormal Ca^{2+} homeostasis in human cardiovascular diseases. More recently, cardiovascular diseases are partly caused by decreased sarcoplasmic endoplasmic reticulum Ca^{2+} ATPase levels or activity. In defective Ca^{2+} uptake, there is a decrease in the relative ratio of SERCA2a phospholamban in these cardiovascular diseases (Rincon, VandenDriessche and Chuah, 2015).

Using of transgenic and gene transfer approaches increasing levels of phospholamban relative to overexpression of SERCA2a in human ventricular cardiomyocytes obtained from patients with cardiovascular diseases. This results in increased SERCA2a pump activity consistent with improved contraction and relaxation velocity. This overexpression of an antisense phospholamban construct or a dominant-negative mutant of phospholamban which is an endogenous muscle-specific inhibitor of the SR (Isner, 2002).

Vectors used in cardiovascular gene therapy

The vectors which are used for cardiovascular gene therapy, should be nonpathogenic, efficiently and transduce target cells (Hulot, Ishikawa and Hajjar, 2016). These vectors elicit a minimal immunogenic response as a result of immunogenicity or unregulated transgene expression. There are two types of vectors which are used in cardiovascular gene therapy. They are viral vectors and non-viral vectors (Hospital, 2002). Basically these vectors serve as "transport vehicles" of the genetic material to the cells. That means the genetic material to be inserted in the target-cells, using either with in vivo or with the ex vivo techniques. These delivery vehicles may be based on plasmid DNA with or without complexing agents, or viral particles (Ponder, 2001).

Viral vectors

Viral vectors consist of genetic material surrounded by a protein based capsid or a lipid envelope which contains the essential viral genes, genes required for replication and structural products. This capsid helps to protect the viral nucleus from degradation in the lysosomes. These viral regions are surrounded by regulatory sequences replaced one or more viral genes with a promoter and coding sequence of interest (Williams *et al.*, 2010). There are some viral vectors, which are useful in cardiovascular gene therapy. They are,

I. Retrovirus

Retroviruses are the most commonly used viral vectors in cardiovascular gene therapy and this retroviral vectors of genetic material were developed in the '80's and firstly used in gene

therapy in cardiology (Gaffney *et al.*, 2007). Retroviruses genome comprised of two copies of identical RNA molecules. This viral vectors have a capacity of transferring genomic material up to 7-10kb. Retroviruses RNA genome is copied into double stranded DNA, which integrated into the host cell chromosome and after that entry into the cells are stably maintained (Fishbein, Chorny and Levy, 2011).

II. Lentivirus

Lentivirus belongs to the retrovirus family. These are another potential vector system for cardiac gene therapy. Lentivirus differs from retrovirus because they have more complex genome and is capable of transducing non dividing cells. This viral vector has a low DNA carrying capacity of 8kb (Nabel, 1995).

III. Adeno virus

Adenoviruses were first described in 1953. They are double strand DNA viruses and they are very suitable for gene therapy because it has high titers achievable and a broad tissue tropsium. There are some characteristics which make adenoviruses attractive for cardiovascular gene therapy. They are,

- They have a broad natural tropism
- Their high nuclear transfer efficiency ensures a rapid onset of transgene expression
- They do not integrate into the host genome
- They can easily be produced in large quantities

IV. Adeno associated virus

Adeno associated virus AAVs are nonpathogenic and small member of the parvovirus family with a 4.7kb single stranded DNA genome. This vectors are known as parvovirus because that require cells to be doubly infected by a helper viruses, such as adenoviruses or herpes simplex virus. AAVs can infect nondividing cells.

Non-viral vectors

Non-viral gene transfer mainly done by using naked plasmid DNA or other forms of DNA/RNA into cells/tissues. This transfer of non-viral vectors to the cells can be performed using ex vivo or in vivo techniques. Non-viral vectors have some special features, which are important to the cardiovascular gene therapy. They are,

- Low immunogenic properties
- Low costs of production
- Low toxicity
- The option for very high organ specificity

1. Cationic lipids

Cationic lipids are anionic DNA plasmids can be mixed with cationic lipids to produce a complex that can then fuse with cell membranes and allow the new genetic materials to enter the cytosol. When the DNA enters the cytosol, then it must be transported to the nucleus, when transcription can occur (Wolfram and Donahue, 2013).

2. Plasmid DNA

Plasmid non-viral vectors are simple closed circular DNA plasmids. Plasmid DNA vectors are important because of their low toxicity and it has safest choice for therapeutic gene transfer. Using only a small number of proteins, plasmid DNA vectors are easily produced large scale. These vectors are used for low density gene transfer (Greenberg, 2015).

CONCLUSION

Cardiovascular gene therapy is emerging as a suitable alternative, with substantial progress in preclinical models of cardiovascular diseases (CVD) (Isner, 2002). The techniques described may be utilized in the future to deliver various genes targeted to combat many different diseases processes, in different animal models, and ultimately to provide feasible gene therapy approaches to human cardiovascular disease (Konishi *et al.*, 2008). This review has covered the area of the vector technology and cardiac gene delivery. The goal of gene therapy is to modify a gene or genetic pathway to provide therapeutic value and prevent or reduce diseases (Hulot, Ishikawa and Hajjar, 2016).

ACKNOWLEDGMENT

The authors wish to thank the International College of Business and Technology, Sri Lanka.

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