Journal of Controlled Release 195 (2014) 110–119

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Functional polymers of gene delivery for treatment of myocardial infarct

Young-Wook Won [a,b], David A. Bull [b], Sung Wan Kim [a,*]

[a] Center for Controlled Chemical Delivery (CCCD), Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT, USA
[b] Division of Cardiothoracic Surgery, Department of Surgery, School of Medicine, University of Utah, Salt Lake City, UT, USA

ARTICLE INFO

Article history:
Received 14 April 2014
Accepted 20 July 2014
Available online 27 July 2014

Keywords:
Myocardial infarct
Gene delivery
Non-viral carrier

ABSTRACT

Ischemic heart disease is rapidly growing as the common cause of death in the world. It is a disease that occurs as a result of coronary artery stenosis and is caused by the lack of oxygen within cardiac muscles due to an imbalance between oxygen supply and demand. The conventional medical therapy is focused on the use of drug eluting stents, coronary-artery bypass graft surgery and anti-thrombosis. Gene therapy provides great opportunities for treatment of cardiovascular disease. In order for gene therapy to be successful, the development of proper gene delivery systems and hypoxia-regulated gene expression vectors is the most important factors. Several non-viral gene transfer methods have been developed to overcome the safety problems of viral transduction. Some of which include plasmids that regulate gene expression that is controlled by environment specific promoters in the transcriptional or the translational level. This review explores polymeric gene carriers that target the myocardium and hypoxia-inducible vectors, which regulate gene expression in response to hypoxia, and their application in animal myocardial infarction models.

1. Introduction

Myocardial infarction (MI) is the leading cause of death in developed nations and one of the most common causes of death in the world. The blockage in coronary arteries by atherosclerosis or thrombus develops ischemic heart disease that includes temporary pain (angina), irregular heart beat (arrhythmia), permanent heart muscle damage (MI), and loss of muscle activity (heart failure) [1]. Cardiac remodeling leading to heart failure is a global and cellular change in ventricular shape and function following chamber dilation, interstitial and perivascular fibrosis. This includes neurohormonal responses, cytokine activation, loss of cardiomyocytes due to necrosis or apoptosis, cardiomyocyte hypertrophy, disruption of extracellular matrix (ECM) and collagen accumulation followed by scar formation [2]. Unfortunately, current pharmacological treatment regimens for myocardial infarction do not reliably limit remodeling of the left ventricle (LV) post-infarction and prevent progression to heart failure [3]. Novel potential treatments, including gene and cell therapies, offer a means to directly treat the pathophysiology underlying the long-term complications of myocardial infarction–loss of cardiomyocytes. The process of remodeling of the left ventricle begins immediately after an acute ischemic insult. The extent of remodeling correlates with the size of the infarct and the decline in cardiac function [4]. Oxidative stress resulting from rapid metabolic changes in the early stages of ischemia plays a crucial role in cardiomyocyte apoptosis and fibrosis of the myocardium [5]. The extent of cardiomyocyte loss in the early stages following an acute MI correlates directly with the subsequent degree of left ventricular remodeling and the decline in cardiac function. This suggests that preventing the loss of cardiomyocytes in the early stages of an acute MI is necessary to achieve long-term efficacy in the treatment of ischemic heart disease.

Since it was first reported in 1972, gene therapy has been a rapidly progressing technology for treating many genetic and acquired diseases including myocardial infarction [6]. The genetic intervention includes (1) overexpression of a target molecule by the introduction of plasmid DNA, (2) a loss-of-function approach by the introduction of RNA interference (RNAi), and (3) correcting deleterious gene mutations/deletions at the genome or primary mRNA level. Neovascularization and the inhibition of apoptosis are considered as good approaches for the sequentially combined gene therapy for ischemic disease. In the early stage of myocardial infarct, reduced oxygen supply and increased reactive oxygen species (ROS) occur in ischemic cardiomyocytes followed by apoptosis. Protecting the cells from apoptosis is the first step, and the second step is to reestablish vasculature through angiogenesis that returns the hypoxic condition back to a normoxic state. DNA, small interfering RNA (siRNA), and micro RNA have been applied to gene therapy. DNA-based gene therapy delivers exogenous plasmid DNA to the cellular nucleus, which encodes a specific gene that enhances the expression of therapeutic proteins. On the other hand, siRNA reduces protein expression by silencing target mRNA in the cellular cytoplasm. However, they must overcome several barriers for successful clinical application such as cell membrane penetration, stability in serum, and safety concerns such as un-controlled gene delivery [7]. To overcome those barriers, DNA and RNA require appropriate delivery vehicles.

* Corresponding author at: Department of Pharmaceutics, Pharmaceutical Chemistry, University of Utah, 20 South 2030 East, Salt Lake City, UT 84112-5820, USA.
E-mail address: SW.Kim@pharm.utah.edu (S.W. Kim).

http://dx.doi.org/10.1016/j.jconrel.2014.07.041
0168-3659/

Various non-viral carriers such as cationic polymers, peptides, liposomes and nanoparticles have been developed and have showed success in the delivery of genes through the cell membrane and into the cell, thus protecting genes from degradation [8].

In 1997, with rationales including a versatile design, no integration into the host chromosome, and non-immunogenic response, research regarding polymeric gene delivery was started [9]. Polymeric carriers are safe for repeated injection, easy to reproduce, and cost-effective, all of which are fundamental considerations in the development of pharmaceutical products [8]. Despite the benefits, they typically show relatively low transfection efficiency and poor therapeutic efficacy compared to virus-mediated gene delivery [10]. Various polymer constructions have been developed to overcome the drawback of polymeric gene carriers. In this review, we describe the use of polymeric gene carriers for treatment of myocardial infarction.

2. Cardiovascular gene therapy

A variety of catheter or surgical approaches for *in vivo* gene transfer into myocardial tissue showed promising results in animal and clinical studies. Specifically, angiogenic gene therapy is of growing interest as an alternative treatment to the conventional protein therapy in the area of ischemic heart diseases. An animal model with chronic ischemic myocardium showed an increase in collateral blood flow and an improvement of cardiac function by the injection of plasmid vascular endothelial growth factor (VEGF) or fibroblast growth factor (FGF) [11,12]. In human clinical trials, the administration of plasmid VEGF into ischemic myocardium through a left anterior thoracotomy resulted in improved heart responses [13]. Similar to VEGF, administration of FGF into epicardial fat also demonstrated an improvement of angina symptoms and an increase in myocardial blood flow [14]. In addition, anti-apoptotic genes and anti-oxidative genes have been widely used in cardiovascular gene therapy. Despite its great potential as treatment to ischemic heart diseases, the transfection efficiency, stability, safety and controlled expression of the therapeutic genes need to be assured.

3. Polymeric gene carriers

A variety of polymer-based gene delivery systems have been developed in the last decade to improve efficacy of therapeutic genes. The polymeric carriers are typically positively charged to bind with the negatively charged cell membrane that blocks gene transfer into cells [7]. Cationic polymers readily condense negatively charged nucleic acids through electrostatic interaction and have thus been widely used as gene carriers [15–17]. The binding affinity between carriers and nucleic acids may decrease the expression of genes because nucleic acids have to be dissociated from the carrier to move to their target location inside the cells [18]. Designing bioreducible polymers with a proton buffering effect for endosomal escape and rapid dissociation in cytoplasm has solved this problem [19]. Bioreducible polymers, or peptides containing internal disulfide bonds in the main chain, at the side chain or in the cross-linker, have high stabilities in extracellular spaces and are rapidly reduced in the cytosol by high intracellular glutathione (GSH) [20]. Polymer reduction can reduce the cytotoxicity of high molecular weight polycations by converting the polymers back into the smaller constitutive subunits and also allow for the release of nucleic acids into the cytoplasm [21]. The use of bioreducible polymers in gene therapy is increasing due to their potential for enhanced transfection efficiency and cytoplasm-sensitive gene delivery.

4. TerplexDNA

A gene delivery carrier was developed that is derived from stearyl-poly(L-lysine) (stearyl-PLL), low density lipoprotein (LDL) and plasmid DNA. TerplexDNA is generated through electrostatic and hydrophobic interactions between LDL, stearyl-PLL, and DNA [22,23]. The PLL component condenses DNA through the interaction of the epsilon-amino group of the lysine with the negatively charged phosphate backbone of DNA [22,23]. The stearyl groups participate in hydrophobic interactions with the core of the LDL molecule allowing integration into the molecule itself [24]. Incorporation of LDL into the polymer gene carrier enhances gene delivery through augmentation of the LDL receptor-mediated endocytosis pathway. LDL receptors exist on the surface of many cells including artery endothelial cells, myocytes, and hepatocytes [25,26].

Compared with Lipofectamine, the TerplexDNA system showed high transfection efficiency without cytotoxicity in a human hepatocyte line (HepG2), murine smooth muscle (A7R5) cell lines [22,23], and bovine aorta primary cell cultures, both vascular smooth muscle cells and endothelial cells [24]. Pharmacokinetic and biodistribution studies revealed that TerplexDNA improved circulation time and prolonged whole body retention. TeplexDNA was less toxic than other cationic polymers because the mechanism of internalization is receptor-mediated endocytosis [23,27]. The main advantage of TerplexDNA might be due in part to the prolonged half-life, which provides the benefit of an increased chance of interaction with the LDL receptor. Another advantage might be the possibility of repeated administration because TerplexDNA is non-immunologic [23,27]. The TerplexDNA system may therefore have applications in the treatment of heart disease in a clinical setting.

4.1. Gene delivery into myocardium

The TerplexDNA system would be beneficial for delivering DNA to the myocardium because the LDL receptors exist on the surface of myocytes [28]. The transfection efficiency of TerplexDNA in rabbit myocardium was 20–100-fold higher than that of naked DNA. This system exhibited more widespread and uniform gene expression near the injection area. In a rat myocardial infarction model, gene transfection was significantly improved without toxicity when compared to naked DNA. TerplexDNA system was developed as an efficient gene carrier that has potential in future clinical applications for the treatment of cardiovascular diseases.

4.2. Gene delivery into primary artery wall cells

Receptor-mediated endocytosis was found to be the main mechanism of TerplexDNA internalization into cells [22,23]. Along with the fact that the cells of the artery wall also express LDL receptors on their cell surface [25], it was hypothesized that TerplexDNA system specifically delivers genes into these cells. This TerplexDNA system was evaluated with these cells for transfecting reporter genes (β-galactosidase and luciferase) or VEGF gene. TerplexDNA system specifically delivered reporter genes and VEGF gene into the cells of the bovine aortic artery wall by receptor mediated endocytosis [24]. The gene transfection by TerplexDNA system was significantly inhibited in the presence of free LDL. This proves that TerplexDNA system is a promising tool for artery wall gene transfer.

4.3. VEGF gene delivery in animal model

The mechanism of left ventricular dysfunction after myocardial infarction is multifactorial and not completely understood [29]. It was reported that the essential role of myocytes that secrete VEGF was to maintain the function of the left ventricle [30]. This finding suggests the autocrine and paracrine roles of VEGF in the maintenance of normal function of the myocardium and the left ventricular extracellular matrix, which can be noted as an important factor to