The Potential of Nanoliposome-Encapsulated PCSK9 Vaccine for Active Immunization Against Atherosclerosis: A Review

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Abstract. Atherosclerosis, a chronic inflammatory disease characterized by lipoprotein deposition in the intima in arteries, is often diagnosed late. This fact has triggered research into immunization strategies for preventing the disease. This review discusses recent studies of immunization using proprotein convertase subtilisin kexin type 9 (PCSK9), with an emphasis on the advantages of the immunogenic fused PCSK-9 tetanus displayed on the surface of liposome nanoparticles and mixed into alhydrogel vaccine (L-IFPTA +) for the prevention of atherosclerosis. The PubMed, ScienceDirect, Nature Publishing Group, Springer, and ResearchGate databases were searched in July 2021 using the keywords atherosclerosis, vaccine, PCSK9, and nanoliposome. Studies that reported the role of the PCSK9 vaccine for passive and active immunization were reviewed. For passive immunization, the PCSK9 vaccine has some drawbacks because it requires regular high doses, is expensive, and is not well tolerated by some people. Active immunization using the PCSK9 vaccine has been tested in preclinical studies. The compounding of the PCSK9 peptide as the B cell epitope to the tetanus peptide in the T-cell epitope generates the immunogenic fused PCSK9-tetanus (IFPT). The improvement of IFPT with an absorbed nanoliposome using Alhydrogel in the form of L-IFPTA + seems to increase the bioavailability of the vaccine and to make it more biodegradable, biocompatible, and less toxic. Active immunization induces long-lasting humoral responses by increasing the production of anti-inflammatory immune cells and provides protection against atherosclerosis by lowering low-density lipoprotein cholesterol level. Compared with the other formulations, L-IFPTA + has the highest therapeutic index. Preclinical studies show that PCSK9-encapsulated nanoliposomes in the form of L-IFPTA + is a potential novel approach for the prevention of atherosclerosis. The effectiveness of active immunization using L-IFPTA + needs further studies.

Keywords: Atherosclerosis · vaccine · PCSK9 · nanoliposome

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1 Introduction

Atherosclerosis is a chronic inflammatory disease that elicits both natural and adaptive immune responses during all disease stages. Inflammation associated with atherosclerosis is characterized by lipoprotein deposition in the intima of arteries. Atherosclerosis has a high prevalence throughout the world and its prevalence is expected to continue to increase. In the United States, more than 82 million people are estimated to have atherosclerosis [1]. Inadequate treatment of atherosclerosis increases the risk of cardiovascular disease. In 2015, an estimated 422.7 million people had cardiovascular disease, which caused 17.9 million deaths worldwide. The increasing prevalence is associated with lifestyle factors, such as a high-fat diet and lack of physical activity [2].

Current treatment guidelines for atherosclerosis recommend the use of medications such as statins, antiplatelet drugs, and antihypertensive agents. However, findings from clinical trials concerned the effectiveness of these medications as the relative risk rate does not even reach 40% [3]. Statin drugs are the gold standard treatment of hypercholesterolemia; however, studies of nonadherence and negative pleiotropic effects are challenges to reaching the target low-density lipoprotein cholesterol (LDL-C) level [4, 5]. In the past decades, immunization has been proposed as a new type of treatment for atherosclerosis. Passive immunization using monoclonal antibodies (mAbs) has been developed and used in clinical settings [6].

Proprotein convertase subtilisin/kexin 9PCSK9 is a liver enzymatic protein that negatively regulates the LDL receptor (LDL-R) and is secreted by hepatocytes. Inhibition of PCSK9 by administration of mAbs to PCSK9 can reduce the levels of LDL-C, very-low-density lipoprotein cholesterol, and total cholesterol by 60% [6]. Several lines of evidence suggest that many cells express the PCSK9 gene [4]. PCSK9 circulates in the blood and acts by binding to the catalytic domain of the LDL-R, which is responsible for LDL-R recycling. Inhibition of PCSK-9 blocks LDL-R recycling, which leads to its lysosomal internalization and degradation. Degradation of the LDL-R can increase the LDL-C level in plasma. Vaccination with mAbs to PCSK9 is expected to reduce LDL-C levels by altering this mechanism [4, 6, 7]. However, the antigenic properties of the PCSK9 peptide are weak, and it has low immunogenicity. Novel approaches are needed to induce a strong antibody response to this autoantigen [4, 8].

Clinical studies have shown the effectiveness of anti-PCSK9 antibodies in treating atherosclerosis. Two mAbs that have been widely studied are evolocumab and alirocumab, which are mAbs to PCSK9 [6, 7]. One of the clinical trials, the FOURIER (Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk) study, is a double-blind, multicenter study of the anti-PCSK9 evolocumab. This trial found that evolocumab reduced LDL-C [9]. Another clinical trial, ODYSSEY (Outcomes Evaluation of Cardiovascular Outcomes After an ACS During Treatment with Alirocumab) was conducted over 12 months in patients with acute coronary syndrome. Based on the National Cholesterol Education Program Third Adult Treatment Panel (NCEP ATP III), the patients were classified into the highest risk category, which recommends reducing the LDL-C concentration to < 70 mg/dL. Trial by Schwartz et al.,2018 the target LDL-C level was 35–50 mg/dL, and alirocumab treatment reduced LDL-C concentration by 62% [10].

The use of anti-PCSK9 antibodies has been approved by the Food and Drug Administration of the United States. Alirocumab was approved in July 2015 and evolocumab in August 2015. This therapy is used by adult patients with familial heterozygous hypercholesterolemia or those with atherosclerosis related to cardiovascular disease who require additional LDL-C reduction after dietary control and statin therapy [4].

Despite the effectiveness of anti-PCSK9 antibodies as passive immunization, its use has several disadvantages [11]. The most serious disadvantage relates to the short lifespan of mAbs, which means that periodic passive immunization is needed at high doses once or twice per month. This makes passive immunization expensive. Periodic immunization at high doses may cause tolerance problems in some patients. This drawback has led to the development of active immunization as a novel approach for treatment of atherosclerosis. Ideally, active immunization induces the production of endogenous antibodies and should be safe, specific, well tolerated, and easy to use [12].

Nanoliposome drug-delivery systems are thought to act as good carriers for peptide antigens. Studies have shown that the nanoliposome PCSK9 vaccine leads to a strong, safe, and functional long-term induction [13]. Research on antigens for vaccination has been performed over the past two decades, but these studies are limited to experimental animals. No reviews of the use of nanoliposome immunogenic fused PCSK9-tetanus mixed into alhydrogel vaccine (L-IFPTA +) in humans has been published. This review discusses the development of active nanoliposome immunization using L-IFPTA + [12, 14].

2 Method

Articles reviewed for this literature review were gathered from several scientific databases, namely PubMed, ScienceDirect, Nature Publishing Group, Springer and ResearchGate using the keywords dyslipidemia, atherosclerosis, vaccine, PCSK9, and liposome. Searching was performed by combining the four keywords using Boolean operations. Consistent with the study objectives, we purposely selected articles published in the past 10 years, in English, and relevant to the specified topic.

3 Result and Discussion

3.1 Passive vs. Active Vaccination Against Atherosclerosis

For more than two decades, researchers have gathered scientific evidence about vaccines against atherosclerosis, for both passive and active immunization [4, 15]. As passive immunization, mAbs (i.e., alirocumab and evolocumab) were approved by the FDA in 2015. Another new mAb, anacetrapib, has entered the third phase of clinical trials [4, 11, 15]. However, some drawbacks have been identified for their use as passive immunization, such as poor drug adhesion and the short lifespan. Passive immunization requires periodic treatment 1–2 times/month at a high dose of 140 mg with a yearly cost of > 14,000 USD. Periodic immunization using a high dose can reduce patient compliance and lead to tolerance [4, 16]. Active immunization is considered to be cheaper, better

tolerated, and easier to use. The adaptive immune response lasts longer than passive immunization, which decreases the dosage and frequency needed [4, 6, 8].

The process of forming an adaptive immune response in active immunization occurs after the internalization of antigen into the body [4, 15]. Active immunization can work in three ways by inducing the formation of neutralizing antibodies, regulatory T cells (Tregs), or both. To form high-affinity neutralizing antibodies, plasma B cells require stimulation by helper T follicular cells from the centrum germinativum in secondary lymphoid organs. The centrum germinativum is the site of affinity maturation, proliferation of mature B cells, replacement, and differentiation of immunoglobulins. Subsequently, Treg regulators formation and autoantigens bind directly to Treg receptors at the same time. Tregs act by reducing the distribution of effector T cells and proinflammatory cytokines, and by producing anti-inflammatory cytokines such as interleukin 10 (IL-10), transforming growth factor beta, and IL-35 [11, 15, 17].

The difference between atherosclerosis vaccines and other vaccines is that the antigens used for atherosclerosis vaccines are self-proteins or autoantigens [4, 8]. These autoantigens can be divided into two types, namely non lipid-related antigens and lipid-related antigens [6]. Nonlipid-related antigens include heat shock proteins, interleukins, B2 glycoprotein I, and C5a. Lipid-related antigens include Ox-LDL, cholesterol ester transfer protein, ApoB100 peptide derivatives such as p210, p2, p143 and PCSK9 [6, 15]. The production of anti-PCSK9 antibodies decrease circulating LDL-C concentration [4, 11]. The antibody produced as anti-PCSK9 induces a strong, specific, long-lasting response and has been shown to be safe [6, 8].

The autoantigens used for the formation of atherosclerosis vaccines are lipid-related antigens because of their direct role in the process of atherogenesis. The most frequently used lipid-related antigens are Apo-B100 and ox-LDL derivatives [6, 11, 15]. Apo B100 and ox-LDL are atheroprotective because they induce production of T cells. However, they also stimulate the production of proatherogenic IgG antibodies [6, 18, 19].

3.2 Production of the Vaccine Containing the Immunogenic Fused PCSK9-Tetanus

The immunogenic fused PCSK9-tetanus (IFPT) vaccine is produced by combining the PCSK9 peptide as the B-cell epitope with the tetanus peptide as the T-cell epitope. The IFPT vaccine is synthesized by high-performance liquid chromatography (HPLC) to a purity grade of > 95%. The cysteine N-terminal residue of IFPT peptide contains thiol group that will react with the maleimide pyrrole group to produce a thioether bond. The peptide binds covalently to the 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N maleimide (DSPE-PEG-Mal) linker, which is used to conjugate immunogenic peptides and nanoliposomes. The required molar ratio between the IFPT peptide and DSPE-PEG-Mal is 1.2:1. The solution is dissolved in chloroform/ Dimethyl sulfoxide (DMSO), stirred at room temperature for 48 h, dried with a rotary evaporator to remove the DMSO/chloroform, freeze-dried, and hydrated with sterile deionized water (pH 7.2) at 30 °C to form micelles of DSPE-PEG-IFPT [16].

The mAb-based IFPT vaccine acts by inhibiting the binding of PCSK9 to LDL-R. The B-cell epitope prepared with immune technology resembles the N-terminal of human PCSK9. Immune systems recognize the peptide mimotope as a foreign object,

which interrupts self-tolerance, and the body produces antibodies specific to PCSK-9. The T-cell epitope is linked to the C-terminal fragment of PCSK9 using the 2-lysine spacer sequence and acts as a carrier [12].

The current research performed in vitro has shown that IFPT increases the response of CD4 + T cells. Anti-PCSK-9 immune response can be tested using enzyme-linked immunoassay. Studies have shown that the humoral immune response to the IFPT vaccine has several shortcomings in terms of its durability. Recently, researchers have sought to develop a vaccination with IFPT using nanoliposomes to increase the amount of IgG, Th2 cells, CD4 + cells, and IL-4 [14, 16].

Liposomes are small round structures that are derived from cholesterol and phospholipids. Liposomes can be used as a carrier compound or to encapsulate medicinal and genetic material. The lipid bilayer comprises lipophilic and hydrophilic portions and protects the compound from degradation. Drug administration using liposome technology can be either passive or active. The passive method is based on the accumulation of the drug at a certain site. The active method involves changing the structure of the target molecule, which can be mAbs. Because of the changes in pharmacokinetics and biodistribution, liposomes with natural lipids are more biocompatible and biodegradable, and less immunogenic toxic. These changes increase the therapeutic index, and the use of liposomes has been approved by the FDA [12, 16].

One limitation of the IFPT vaccine is that it requires an adjuvant, called nanoliposomes, to increase the bioavailability. Nanoliposomes can maintain the IFPT level in the blood and stimulate immune responses to decrease toxicity. The surface of the nanoliposome used as an adjuvant component is negative, and this property increases the response of antibodies to PCSK9 in mice. The purpose of nanoliposomes in IFPT vaccines is to improve the efficiency of the mechanism of action [16, 20].

3.3 Potential of the Anti-PCSK9 Nanoliposome Vaccine in Atherosclerosis

Momtazi-Borojeni et al. (2021) reported that nanoliposomes are synthesized by inserting IFPT into the surface nanoliposome using DSPE-PEG-Mal lipid (L-IFPT) and absorbed onto Alhydrogel (L-IFPTA +). The size of L-IFPTA + is 120–155 nm and the polydispersity index is < 0.1, which means it has high homogeneity [11, 16, 21].

The use of the L-IFPTA + vaccine has been shown to increase the response to antibodies above that for other types of vaccines after four immunizations in a hypercholesterolemia rat model [12]. Another study showed that, after 16 weeks of immunization with L-IFPTA +, the antibody levels of the vaccine can stimulate the response of the humoral immune system and decreased LDL levels up to 127.7 ± 7.4 mg/dl [16]. The L-IFPTA + vaccine lowered plasma PCSK9 level by 40%, by increasing the levels of LDL-R on the surface of the liver and by decreasing the binding of PCSK9 to the LDL-R by 70% in vitro compared with placebo control [13].

Immunization of mice with L-IFPTA + also significantly decreases plasma LDL-C concentration, prevents atherosclerotic lesions, and has anti-inflammatory effects [12]. The formulation of the anti-PCSK9 vaccine triggers the production of the antibody PCSK9 that decreases the plasma LDL-C level directly. Three studies have reported that 43% of mice given L-IFPTA + immunization had degree I lesions, whereas 71.5%

of control mice had severe degree III or IV lesions [14]. Analysis of flow cytometry in spleen cells indicated that the L-IFPTA + vaccine is immunologically safe and improved the response as an anti-inflammatory. In l-IPFTA + vaccinated vaccine, the anti-inflammatory effect was shown by 1.76- and 4.9-fold increases in the number of Th2 cells and IL-4 cytokine, respectively. The responses of Th2 cells and IL-4 were related to each other and participated in fat cells by increasing M2 type macrophage cells as an anti-inflammatory agent [13].

4 Discussion

PCSK9 inhibition is recognized as the most promising LDL-lowering approach for the treatment of hypercholesterolemia and cardiovascular disease. Clinical research on cardiovascular events and methods to reduce LDL-C level using PCSK9 mAbs in the form of alirocumab and evolocumab have led to their approval as PCSK9 inhibitors [16]. However, there are some limitations to this therapy such as the need for repeated administration and cost. Despite these limitations, active vaccination with long-term effects can provide a safe alternative for hypercholesterolemic immunotherapy. The studies published so far are limited to preclinical trials, and no studies have tested vaccination in humans [12, 16].

PCSK9 is one peptide that can be used for active vaccination. However, PCSK9 has some weaknesses, mainly its low immunogenicity. Trials have found new ways to increase the efficacy of the PCSK9 active vaccine by using nanoliposomes or the L-IFPTA + vaccine. Momtazi-Borojeni et al. [13] reported that the L-IFPTA + vaccine given to atherosclerosis-induced mice reduced PCSK9 levels in plasma. This was also accompanied by an increase in LDL-R levels on the liver surface and decrease in PCSK9 binding to LDL-R in vitro compared with controls. That study also showed that mice given the L-IFPTA + immunization showed grade I lesions, whereas control mice showed severe grade III or IV lesions. Findings from this study suggest that the L-IFPTA + vaccine may be an effective vaccine against atherosclerosis [13].

Few studies have been published on the use of L-IFPTA + vaccine in the active immunization against atherosclerosis [12]. A limitation of this review is that the evaluation was limited to English language journals and we may have missed articles published in other journals that may contain new information.

5 Conclusion

Preclinical studies confirm the potential of PCSK9-encapsulated nanoliposome in the form of L-IFPTA + as a preventive treatment for atherosclerosis. As a novel approach for preventing atherosclerosis, active immunization using L-IFPTA + needs further studies to evaluate the efficacy of the vaccine in humans.

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