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Vaccines Directed Against Proprotein Convertase Subtilisin/Kexin Type 9

ABSTRACT

KEY WORDS: Vaccines, PCSK9, primary prevention, animal models, LDL

Despite progress in primary and secondary prevention, atherosclerotic cardiovascular disease remains one of the leading causes of mortality. Long-term exposure to elevated levels of low-density lipoprotein (LDL) cholesterol is a significant risk factor for its development. Various treatment strategies for hypercholesterolemia exist; however, their success is limited. Treatment with tested and registered humanized monoclonal antibodies against proprotein convertase subtilisin/kexin type 9 (PCSK9) is not widely available due to high costs. One potentially effective treatment currently under preclinical and clinical investigation is the development of vaccines against PCSK9. This could ensure a long-term reduction of total and LDL cholesterol in an effective and affordable way. Preclinical research on animal models and a smaller clinical study indicate that these vaccines induce a significant, long-lasting antibody response that effectively lowers LDL and total cholesterol levels without significant side effects. Larger clinical phase II and III studies are still needed to further elucidate the safety and efficacy of PCSK9 vaccines. In this article, the current knowledge in the field is briefly summarized.

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INTRODUCTION

Atherosclerotic cardiovascular disease is a degenerative inflammatory process characterized by the accumulation of lipids and inflammatory cells within arterial walls *(1)*. Lipid-rich deposits, known as plaques, gradually narrow the arteries, causing ischemia or, in the case of plaque rupture, thrombosis. The interplay between elevated levels of cholesterol in low-density lipoproteins (LDL) and other cellular processes (endothelial cell dysfunction, formation of foam cells, activation of various immune cells) in the arteries leads to the gradual progression of atherosclerosis throughout life *(1)*. The proprotein convertase subtilisin/kexin type 9 (PCSK9) molecule influences cholesterol homeostasis by binding to the epidermal growth factor-like domain A (EGF-A) on the LDL receptor in hepatocytes, initiating the degradation of the LDL receptor and thereby reducing the serum clearance of LDL cholesterol *(1)*. Several genotypes related to PCSK9 and its ability to influence cholesterol homeostasis affect the phenotype: mutations that enhance PCSK9 function cause a rare form of familial hypercholesterolemia, while loss of function (LOF) mutations that reduce PCSK9 function result in lower levels of LDL cholesterol and protect against atherosclerotic cardiovascular disease *(2)*. People who carry homozygous or two heterozygous LOF mutations have undetectable PCSK9 levels and exhibit very low LDL cholesterol levels and are protected from developing atherosclerosis and its atherothrombotic complications *(3)*. Current therapeutic strategies for treating atherosclerosis involve lowering LDL cholesterol levels using statins, which is an effective approach. However, many individuals, particularly patients with familial hypercholesterolemia, do not respond to or tolerate such therapy. PCSK9 emerges as a natural target for preventing hypercholesterolemia, as it has been shown that people with heterozygous LOF mutations live without serious side effects *(4)*. Large clinical studies have demonstrated that patients receiving PCSK9 inhibitors in the form of fully human neutralizing antibodies significantly reduced their LDL cholesterol levels *(4, 5)*. Despite the effectiveness of monoclonal antibodies, there are several drawbacks to this type of treatment. First, antibodies have a relatively short half-life *in vivo*, and second, the therapy, which requires frequent dosing once or twice a month, is expensive *(4)*. A PCSK9 vaccine could represent a cheaper but still safe alternative for effectively lowering LDL cholesterol and preventing atherosclerotic complications.

VACCINES AND IMMUNE RESPONSE

In recent years, we have witnessed rapid progress in the development of vaccines extending beyond the prevention of infectious diseases. The functioning of the immune system can be divided into innate and acquired immune responses. Upon encountering a foreign antigen, antigen-presenting cells (APC) take it up from the environment, degrade it into shorter peptide molecules via endosomal pathways, and present these within the framework of the major histocompatibility complex (MHC) I or II to cluster of differentiation (CD) 8-positive and CD4+ lymphocytes *(6)*. This is the innate immune response. When foreign immunogenic molecules are recognized and encountered by B lymphocytes, a signalling pathway is triggered, causing the production of foreign-specific B lymphocytes, their maturation, and differentiation into plasma cells that secrete foreign-specific neutralizing antibodies. This is the so-called acquired immune response: neutralizing antibodies deactivate the antigen and mark it for elimination before it can cause damage to the organism, while remaining antibodies act as a part of the immune response »memory« *(6)*. For the

development of vaccines against external antigens, it is desirable that the vaccines induce a specific and targeted immune response based on the development of specific neutralizing antibodies and/or cytotoxic T lymphocytes, while this response is maintained for potential future infections *(6)*. To prevent an autoimmune response to the organism's own (endogenous) proteins, immune cells are subjected to negative selection during maturation. This does not mean that the formation of antibodies against endogenous proteins and/or their fragments cannot be triggered; this can be ensured provided there is stimulation of B lymphocyte activation by helper T lymphocytes *(6)*. If we simultaneously prevent the formation of CD8+ cytotoxic lymphocytes, which would cross-reactively attack cells producing the target endogenous protein, we can ensure the safety of the vaccine against endogenous targets without causing systemic inflammation in otherwise healthy tissues of the organism.

Vaccines inducing antibody response against proprotein convertase subtilisin/kexin type 9

Vaccines against PCSK9 stimulate the production of neutralizing antibodies that prevent the binding between the PCSK9 molecule and the LDL receptor. Several preclinical studies indicate that this could be an effective and safe way to lower LDL cholesterol. Galabova and colleagues used short peptides composed of 8–13 amino acids from the N-terminal part of human PCSK9, conjugated to a carrier protein that does not elicit self T cell responses *(7)*. Upon adding an aluminium adjuvant, they demonstrated the induction of antibodies against PCSK9, and a reduction of LDL and total cholesterol in laboratory rats. They also showed a reduction in total and LDL cholesterol in mice, which persisted even after six months compared to controls. No cytotoxic T cell response was observed *(7)*. The same group used the AFFITOPE technology to create peptides similar to PCSK9, thereby producing specific antibodies against PCSK9 *(8)*. Using this technology, they developed two vaccines: AT04A and AT06A, which were used in a phase I clinical trial to investigate safety, tolerability, immunogenicity, and the impact on LDL and total cholesterol concentration in healthy subjects. The single-blind, randomized, controlled trial included 72 subjects with an average LDL cholesterol value of 3.0 mmol/L. Subjects were randomly divided into three equal groups that received the AT04A, AT06A, or placebo vaccine for four weeks *(9)*. This was followed by a second phase in which subjects underwent regular check-ups without receiving therapy. In the final phase, they received a booster dose of the vaccine or placebo. The study concluded with 49 subjects, with both vaccines being safe and immunogenic, but only the AT04A vaccine showed a statistically significant reduction in LDL and total cholesterol levels. Further research with the AT04A vaccine is planned *(9)*.

CONCLUSION

Vaccines targeting PCSK9 appear to be potentially successful, safe, and cheaper alternatives to the established therapy with fully humanized monoclonal antibodies against PCSK9. In some studies, they successfully reduced total and LDL cholesterol levels and decreased the growth of atherosclerotic plaques. Peptide vaccines AT04A and AT06A were the only ones tested in humans in a phase I study, examining safety, effectiveness, and observing changes in total and LDL cholesterol levels. Only the AT04A vaccine proved to be safe and effective, but in a small number of subjects. As possible alternatives to peptide vaccines, nanoliposomal vaccines and virus-like particle vaccines are being tested. These have indicated the potential for safely and effectively lowering LDL and total cholesterol

by inhibiting PCSK9 molecules in animal models. Vaccination continues to be an interesting and potentially effective addition to established, evidence-based therapy, primarily limited by the discontinuation of prescribed oral therapy and the high cost of biological drugs *(4)*. Research conducted so far indicates moderate treatment effectiveness in animal models, but vaccine improvements and the successful completion of all phases of clinical trials will be necessary before use in humans.

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