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Research Progress of Targeted Therapy in Atherosclerosis Based on the ApoA- I

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Abstract:

The chronic inflammatory disease atherosclerosis, is caused by the buildup of plaque within the arteries. This may result in the narrowing and stiffening of the lumen. Current studies have shifted towards innovative treatments that target specific proteins central to atherosclerosis development. The subject of focus in this article is ApoA-I, a crucial component in the mechanism of reverse cholesterol transportation. ApoA-I's high effectiveness in interacting with ABCA1 and ABCG1 receptors on membranes facilitates the extraction of phospholipids and cholesterol from foam cells. In vivo experiments suggest that ApoA-I could serve as a novel therapeutic target for coronary atherosclerosis, but its extended effects require further investigation. The last step in the advancements of ApoA-I research would be clinical and human-based trials. As of currently, no human treatment experiments have been carried out, and the effect loss caused by a rejection reaction cannot be ruled out, and more research must be done before it is medically used.

Keywords: Targeted therapy; atherosclerosis; ApoA-I; chronic inflammatory disease.

1. Introduction

Atherosclerosis is a chronic inflammatory disease of the arteries that causes plaque to build up and harden inside them. This plaque buildup can narrow and stiffen the arteries, which can lead to health complications. It is a slow, progressive disease that can start from childhood in humans, but can also progress rapidly through time. Plaque consists of a combination of cholesterol, calcium, fatty substances, cellular debris, and fibrin present in the blood. The formation of plaque initiates when the arterial inner walls are compromised by factors such as elevated blood pressure, high blood glucose levels, excessive cholesterol, or smoking. In response to arterial wall damage, the body triggers an inflammatory reaction where immune cells migrate to the injury site and release chemical mediators, promoting the accumulation of cholesterol and cellular debris. This accumulation attracts white blood cells, which engulf the cholesterol, leading to the development of aggregates that eventually contribute to plaque formation [1].

The treatment method to be discussed in this paper will be Apolipoprotein-I (ApoA-I). ApoA-I is the main protein component of high-density lipoprotein (HDL), often referred to as "good cholesterol." ApoA-I plays a crucial role in reverse cholesterol

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transport, a process where excess cholesterol is transported from peripheral tissues back to the liver for excretion. Increasing levels of ApoA-I or enhancing its function can help reduce atherosclerosis by promoting cholesterol efflux and reducing lipid accumulation in arterial walls [2]

Nowadays, because of the rapidly growing number of patients of atherosclerosis, humans devote themselves to looking for a new-type, more efficient and more effective treatment, targeting ApoA-I to treat the disease, instead of surgery or other physical therapies like catheter. According to the pathological mechanism and then find the suitable protein as a carrier to treat atherosclerosis. Each protein has its own specific amino acid and gene sequence, so research can take advantage of this point and modify the gene to better achieve therapeutic purpose. Based on the current research results: now exist three main antigens: PCSK9, HSP65 and Apo8 and this antibodies to PCSK9 have been confirmed by many experiments and the targeted PCSK9 is very safe in clinical trials, people can use this to improve and develop the targeting ApoA-I to treat atherosclerosis [3]. If people could have this new type of treatment, then people can significantly reduce the pain and other drawbacks or malpractice caused by treatments like surgery and catheters. On the other hand, this method is simpler and easier to operate, increasing efficiency of the whole process, as well as improving the chances of patient survival.

2. Targeted Therapy and Atherosclerosis

2.1 Pathological Mechanism and Treatment

2.1.1 Pathogenesis of aherosclerosis

The development of atherosclerosis initiates with the injury to the endothelial cells that coat the blood vessels in the body. The damage to endothelial cells may stem from hypertension, smoking or elevated LDL cholesterol levels. Increased permeability of the arterial wall is a consequence of endothelial cell damage, enabling the passage of lipoproteins and immune cells, including WBC's.

In typical conditions, the WBC's flow unrestricted within the blood vessels of the arteries without adhering to the endothelial cells. When the endothelial cell is harmed, it releases adhesion proteins that draw white blood cells to it. Through diapedesis, these white blood cells alter their shape to move between endothelial cells.

Once inside the arterial wall, LDL cholesterol particles accumulate within the tunica intima. The LDL particles are subsequently exposed to free radicals and reactive oxygen species and the interaction between the free radicals and LDL cholesterol leads to the formation of oxidized LDL (oxLDL). This oxidized form of LDL is highly atherogenic, because it acts as a potent pro-inflammatory agent and initiates an inflammatory response within the artery and signals monocytes to the site of injury. These monocytes differentiate into macrophages, which then engulf the oxLDL particles, becoming foam cells. Foam cells contain an abundance of oxLDL particles, causing the cytoplasm to resemble foam due to the high lipid content. The accumulation of these foam cells results in the development of a fatty streak, which represents the initial detectable indication of atherosclerosis [4]

During the advancement of the illness, the initial fatty deposit transforms into a more intricate atherosclerotic lesion, with smooth muscle cells moving from the middle layer to the inner layer and starting to multiply. The cells release substances such as collagen that aid in creating a protective layer of fibrous tissue around the central fatty region. This plaque causes the lessening of arterial lumen diameter and causes the vessel to harden, reducing its elasticity and impairing blood flow.

As time passes, atherosclerotic plaques may lose stability, especially when the fibrous cap is thin and prone to breaking. The rupture of a plaque results in the exposure of thrombogenic material below, initiating the development of a blood clot. A blood clot has the potential to obstruct the artery either partially or entirely, leading to sudden cardiovascular incidents like a heart attack or stroke.

When a heart attack or a stroke happens, it will already be a very late stage for the targeted treatment with ApoA-1. The therapy mainly targets the inflammatory stage of atherosclerosis, such as with its anti-inflammatory properties. Some of these properties include the promotion of cholesterol efflux, and the reduction of oxidative stress through the removal of excess cholesterol from cells. Other methods include reducing oxLDL levels through anti-oxidant actions, which will be discussed further below.

2.1.2 Targeted therapy for atherosclerosis

ApoA-I plays a significant role in human physiological activities, such as cholesterol transport and lipoprotein composition[4]. Meanwhile, clinical trials have proved that the abnormal expression of ApoA-I can play a role in CVDs and malignant tumors [5]. Therefore, according to the important role of ApoA-I in the disease, ApoA-I can be used as a new target for the treatment of coronary atherosclerosis, and the role of ApoA-I can be exerted from a new microscopic method.

Atherosclerosis is a chronic vascular disease characterized by inflammation, triggered by the deposition of lipids. The inflammatory response marks the initial stage of this condition. ApoA-I is thought to play a role in modulating both

inflammatory and immune responses [6]. The gene encoding ApoA-I shares a common evolutionary origin with the genes for other proteins, including ApoA-II, ApoA-IV, ApoC-I, ApoC-III, and ApoE, all of which exhibit the characteristic α-amphipathic helix [7]. The regulation of the ApoA-I promoter is a crucial step in this process, involving multiple factors and proteins. Among these, hepatocyte nuclear factor 4 (HNF4) can both activate and inhibit the ApoA-I promoter. Additionally, Liver Receptor Homologue 1 (LRH1) and ApoA-I Regulatory Protein 1 (ARP1/NR2F2) are involved in modulating the activation and repression of the ApoA-I promoter [8].

Once ApoA-I is translated within cells, the N-terminal signal peptide is cleaved, resulting in a mature, lipid-free protein. This protein is composed of 243 amino acids and has a molecular weight of around 28 kDa [9]. Typically, ApoA-I constitutes about 70% of the protein content in HDL, making it the predominant component of HDL. Therefore, based on that condition, the current idea is to aggregate the treated ApoA-I into the macromolecular protein HDL. Previous studies have proved that anti-inflammatory effect of ApoA-I is accomplished through a variety of mechanisms and can be used in various research [6]. This anti-inflammatory mechanism is a complex process, are presented in the kind of inflammatory cytokines like TNF and IL-1β inhibit the production of ApoA-I in hepatocytes and then enhance the expression of serum amyloid A (SAA). The level of SAA is increased so that it can replaces ApoA-I as the major molecular component of HDL [10]. However, at the same time, ApoA-I interferes with the special polymerization and incorporation into the membrane and contributes of C9 and then with the previous step, it can inhibit C5b-9, the formation of the terminal attack complex of the complement, to complement clearance [11]. Clearing the complement prevents the activation of the complement system and triggers a series of biological defense responses, thereby preventing inflammation.

ApoA-I is the main component of HDL, characterized by its highly flexible structure, which allows it to transition between lipid-bound and lipid-free states. This flexibility enables ApoA-I to interact effectively with the ABCA1 and ABCG1 receptors on cell membranes, playing a crucial role in the clearance of cholesterol and foam cells. ApoA-I is an essential factor in the reverse cholesterol transport (RCT) process, with its function being critical across multiple steps of this pathway. During RCT, ApoA-I facilitates the formation and stabilization of HDL particles, which in turn promote the clearance of peripheral cholesterol and its uptake by the liver. Subsequently, HDL interacts with the ABCA1 receptor, ultimately activating LCAT and binding to SR-B1 to exert its physiological functions [12]. Research has shown that introducing the ApoA-I gene into mice significantly inhibits the expression of TLR4 in endothelial cells and regulates the expression of ICAM-1 and VCAM-1. These molecules are crucial in the transendothelial migration of leukocytes during the inflammatory response, highlighting ApoA-I's important role in suppressing inflammation [13]. Additionally, under chronic inflammatory conditions, the structure and function of ApoA-I may be altered. For instance, mutations such as K107del, L144R, A164S, and L178P can lead to changes in the conformation and thermodynamic stability of ApoA-I, thereby impairing HDL function [14]. These mutations may not only affect the anti-inflammatory properties of ApoA-I but also compromise the structure and function of HDL. Targeting the restoration of ApoA-I function or the stabilization of HDL particles could offer new avenues for treating conditions associated with cholesterol dysregulation and chronic inflammation. Moreover, enhancing the anti-inflammatory properties of ApoA-I might improve outcomes in inflammatory diseases and support cardiovascular health.

Under chronic inflammatory conditions, ApoA-I amyloidosis is the main way of ApoA-I mutation. In addition to several trypsin-digested peptides derived from the wild-type ApoA-I, there are DSGRDYVSQFK, DYVSQFK, two following peptides were detected. These two peptides corresponded to wild-type ApoA-I chips at correlated sites 24-34 and 28-34, except for K34 [15], 100% of the protein sequence coverage in this process is provided by the resulting peptide chips [16]. The aggregation of 9-11 kDa N-terminal fragments of Apolipoprotein A-I (ApoA-I) into fibrils is implicated in organ damage, particularly affecting the heart and contributing to atherosclerosis. This condition is exacerbated by mutations in ApoA-I, such as the non-amyloidogenic L159R variant, which impairs high-density lipoprotein (HDL) maturation and increases the risk of atherosclerosis [17].

Research indicates that various ApoA-I mutations have been linked to disease, with L159R and L170P causing significant structural disruptions in free ApoA-I. These mutations reduce the stability of the 4-helix bundle segments while enhancing the stability of the C-terminal tail. Specifically, the L159R and L170P mutations decrease protein ordering in the central region of ApoA-I while increasing it in the C-terminal tail, establishing them as critical factors in the pathology [18].

Additionally, there is a hypothesis suggesting that the free forms of L159R and L170P may have an increased tendency to form dimers, potentially further contributing to their pathogenicity. This dual mechanism of structural perturbation and dimerization could play a significant role in the development of atherosclerosis and related cardio-

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vascular diseases [16].

Represented by L159R, the presence of low levels of plasma ApoA-I L159R may be due to errors in folding the mutant protein during synthesis and its entry into the quality control pathway in subsequent reactions [19], and the low levels of L159R is the induction factor of all subsequent adverse reactions. In reverse cholesterol transport, HDL incrementally increases in size upon increasing lipid cargo [20]. ApoA-I mutations have a great influence on HDL, protein perturbations are caused by mutations and are propagate to distant sites on lipoproteins, however, the current study presented that compared with free protein in the structure the mutations had much smaller effects on the configuration of the lipid-bound [16]. Although the effect is not so great compared to free protein, but if people can improve the occurrence of mutations, as ApoA-I flow increases, HDL will also become active in the human body or arteries again and improve the disease status.

The normal form of this protein in humans is a high-resolution crystal structure model of the full-length lipid-free ApoA-I monomeric with the C-terminal of ApoA-I truncated [21]. But at the same time, if patients have this disease, the C-terminal domain in the protein during this process is still missing, although the patient needs it and it will also play an important role in this process. Because this important structure have two main functions, namely: decreasing of HDL and cholesterol efflux will caused by truncation mutations like Mytilene at the C-terminal domain and besides, it also helps with this theory: HDL formed by dimerization can be governed by the H5 region through three states [21]. So precisely, this important structural defect affects the treatment of the condition.

Studies have established a notable correlation between SNPs in PCPE2 and levels of plasma HDL cholesterol. This suggests that PCPE2 plays a protective role against atherosclerosis and is a crucial component in the reverse cholesterol transport mechanism associated with HDL.

PCPE2, a glycoprotein found in the extracellular matrix, is involved in modulating HDL metabolism. In experimental models, PCPE2 deficiency has been linked to increased HDL concentrations, which paradoxically does not confer the expected protective effects against diet-induced atherosclerosis. Instead, these mice exhibited greater lipid accumulation in arterial tissues, indicating that while elevated HDL levels are present, their functionality may be compromised [22]. PCPE2 has an important role in keeping HDLs molecular level and morphology stable and does not cause more serious blockage. PCPE2 controls SR-BI-mediated HDL-CE uptake and then achieves the purpose.

2.2 Problems and optimization improvement

The use of these PCPE2 with ApoA-I as a targeted drug for treatment is more convenient and rapid than traditional surgical intervention, while reducing the risk and harm caused by physical therapy. Traditional treatments for atherosclerosis, such as surgery and catheters, both use physical interventions to improve blood pressure and speed of blood flow, ultimately treating the disease. The treatment period of these methods is long, the cost of manpower and material resources, and the instability is large. The approach with targeted therapy is simpler, and the patient has more flexibility and less pain. And using this method to supplement the required proteins and related biomacromolecules has the potential to treat the disease at its root. However, this treatment method also has drawbacks, so far, no relevant human treatment experiments have been carried out, and the effect loss caused by rejection reaction and other interference cannot be ruled out after the drug enters the human body. So this method is relatively unstable.

For the current situation, people can take the next step to promote and optimize this targeted drug. Experiments can continue until the drug stabilizes in mice and can be put into clinical trials. While advancing, drugs should continue to be optimized and therapeutic improvements targeted at the root causes of the disease.

3. Conclusion

In summary, the document delves into the targeted therapy for atherosclerosis using ApoA-I as an alternative to traditional surgical procedures. ApoA-I plays a crucial role in facilitating reverse cholesterol transport by effectively interacting with ABCA1 and ABCG1 receptors on cell membranes to eliminate phospholipids and cholesterol from foam cells. As stated in the introduction, society is slowly opting for a more efficient and more effective treatment method, instead of the more traditional methods such as surgical procedures, which could have potential side-effects. ApoA-I will also be a quicker and easier treatment to use, requiring less personnel training, which could be a determining factor in the treatment becoming widespread. The paper delves into the various characteristics of ApoA-I treatment, while also addressing the potential impact of ApoA-I mutations like K107del, L144R, A164S, and L178P on the protein's structure and stability, potentially reducing treatment efficacy by half. These possibilities should still be researched further in vivo, before clinical trials are conducted. Another aspect of ApoA-I therapy that should not be neglected is its long term effects. Due to the targeted therapy being relatively new,

no data is available for the effectiveness or safety of the treatment in 5 year's time or 10 years' time etc. The last step in the advancements and future of ApoA-I research would be human-based trials. The possibilities of an autoimmune response caused by a rejection reaction cannot be ruled out, and more information regarding bodily response to ApoA-I must be properly tested. There is still a lot of work that must be done before ApoA-I is medically used, but if possible, it may very well be the next globally-distributed cure to atherosclerosis, due to it's treatment method.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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