

The Possibility of Using Proteins as a Treatment of Atherosclerosis

Xinran Ma

The Spence School, New York, NY, United States

xinranma354@gmail.com

Abstract. Atherosclerosis is a type of heart disease in which most patients either have coronary disease or carotid disease. Patients with atherosclerosis have narrowed blood vessels due to the formation of plaque or the condensation of fat molecules in the blood. The death rate from atherosclerosis is not extremely high, but atherosclerosis can easily lead to fatal diseases like heart attack and stroke. Atherosclerosis can be prevented by keeping a healthy lifestyle and taking medicines, and severe cases can be treated with surgeries. Protein treatment is not widely used for the treatment of atherosclerosis, but some proteins like prosaposin and HMG-CoA display their essential role in the synthesis of plaque. Prosaposin affects the inflammation of macrophages in the cell by affecting mTOR and S6K1 signaling passageways. Inflammation is the root cause of atherosclerosis, and decreasing inflammation can lead to a decrease in plaque. HMG-CoA is another protein that can affect cholesterol synthesis, which is crucial in plaque formation. HMG-CoA reductase inhibitor, which can be called statin, blocks the HMG-CoA from functioning and decreases the rate of cholesterol formation. The possibility of using prosaposin and HMG-CoA as a protein treatment is proven, and future experiments could determine the method to apply the treatment in clinical trials.

Keywords: Atherosclerosis, prosaposin, HMG-CoA, protein treatment, macrophage.

1. Introduction

Atherosclerosis is a type of cardiopathy that is widespread throughout the world. Atherosclerosis can lead to severe diseases like heart attacks and has a high death rate. Diseases related to atherosclerosis is one of the main cause of death each year in the United States [1]. Millions of people around the world are concerned by this disease. Atherosclerosis refers to the accumulation of fatty and fibrous material in the inner layer of arteries [1]. After the accumulation of plaque in blood vessels, the space inside the blood vessel would be narrowed, then the amount of oxygen blood could deliver would decrease. Both patients with healthy and unhealthy lifestyles have the possibility of getting atherosclerosis. Atherosclerosis can be caused by various reasons, like addiction to smoking and drinking, unhealthy high or low blood cholesterol levels, and high blood pressure. Patients with atherosclerosis would feel shortness of breath, unusual heartbeat, and difficulty with speech, which is due to the lack of oxygen. People with those symptoms can be diagnosed with atherosclerosis by taking blood tests, electrocardiograms, or heart imaging tests [1]. Currently, the world has a few treatments for atherosclerosis but all with different disadvantages. Besides the healthy lifestyle that patients practice daily, a few types of medicines that can lower cholesterol, improve artery health, and prevent atherosclerosis are also used.

Atherosclerosis can lead to dramatic changes in a patient's life. People's economic status and social impact would also be a factor in the severity of atherosclerosis. People with atherosclerosis should decrease their pressure and have more control over their lives, but people with higher status would be comparatively easier to reach this goal. In a research paper called "Socioeconomic Status and Carotid Atherosclerosis", the experiment shows the relationship between the patient's income, occupation, education experience, and their intima-media thickness (IMT) [2]. The IMT is a method to measure the thickness of the tunica intima and tunica media. Those are the inside two layers of the artery. If intima-media thickness in artery increases, the inner space of the artery would decrease, and atherosclerosis begins to form.

Protein treatment of atherosclerosis is still not widely used and the world does not pay much attention to it. However, a protein treatment would be a beneficial treatment due to the little side effect it has, and to regulate the patient's protein to treat their disease lessen the risk in it. A protein treatment would have a better effect on the limitation of the formation of plaque, which would affect atherosclerosis as a result. A few proteins have been tested as effective in treating atherosclerosis, but all the protein treatments are still in the experimental process.

2. Atherosclerosis

Atherosclerosis happens in the arteries due to the accumulation of plaque. Atherosclerosis is not only a type of heart disease, but it can happen in any place an artery exists, the heart, arms, legs, and brain. However, atherosclerosis mainly exists in two types of arteries, which are coronary arteries and carotid arteries.

2.1. Coronary Artery Disease

Coronary artery disease is located in the heart. One major function of the heart is to pump the blood throughout the body and control a normal heart rate and blood pressure. Therefore, when the blood vessel becomes narrowed due to the accumulation of plaque, less blood can be pumped each time, and less blood would transfer through the body to provide oxygen. Atherosclerosis in the heart requires a few surgeries to treat it. The first treatment is coronary artery bypass grafting (CABG), which is an open-heart surgery in which the doctor removes a vein from another place in the body and attaches it to the blood vessel near the blockage. When the narrowed blood vessel cannot deliver enough blood, the vein attached to the blood vessel can help. However, this surgery has an underlying risk in it. The patient has a high possibility of bleeding during or after the surgery. Blood clots can also cause heart attack, stroke, or lung problems. After the surgery, the patients might get an infection at the incision site. The second method to treat atherosclerosis is angioplasty and coronary stent placement. This surgery delivers a balloon and stent into the narrowed vessel, and the balloon is inflated to push the stent outward, and then push the blockage to the side. After the stent is enlarged, the stent will stay in its current form and the balloon will move away. The stent placement treatment has the risk of damaging the artery and causing bleeding [3]. The formation of atherosclerosis in the heart is due to inflammation that accumulates plaque. Plaque is a type of fatty deposits, and it forms due to foam cells, which are formed by macrophages. A macrophage is a type of white blood cell that engulfs bacteria in the immune system. When macrophages detect injury in tissues, the inflammation process will begin. Inflammation is an immune response to harmful stimuli, like toxic particles, radiation, and dead cells. Inflammation is essential to the health of people and is vital to human lives. However, even though inflammation checks people's health status, chronic inflammation could start the immune system to attack healthy organs and tissues. If chronic inflammation still stays untreated, it will increase the risk of getting diseases like atherosclerosis, diabetes, and cancer [3]. The plaque in the blood vessel is formed by foam cells that accumulate through the inflammation process to form solid lipid cores and fibrous surfaces, that is the whole formation process from inflammation to plaque. Macrophage has three functions in the inflammation process, which are antigen presentation, phagocytosis, and immunomodulation [4].

2.2. Carotid Artery Disease

Carotid artery disease is a type of atherosclerosis that happens in the neck. The two common carotid arteries located on the left and right sides deliver oxygen-rich blood to our neck and brain, so a carotid artery disease can lead to a lack of oxygen in the brain, which could be a danger for the ability to understand speech and even speak. For the treatment of carotid artery disease, the main surgery is called carotid endarterectomy. In this one to two-hour surgery, the doctor would make seven centimeters to ten-centimeter cut on the side of the neck and make a small cut on the artery where the plaque is located. Then, the plaque would be removed, and the surgery would be finished.

The carotid endarterectomy is a comparatively easier surgery in which the patients only need to stay in the hospital for forty-eight hours [4]. The risks of this surgery are strokes and heart attacks which can all lead to death. The survival rate after this surgery is 78.2% after five years and 45.5% after ten years [3]. Besides the accumulation of plaque due to inflammation, plaque can also accumulate because of the deposition of fat, cholesterol, and cellular waste product in the blood. Those depositions led to the carotid bifurcation becoming the most common place to have plaque accumulated. Plaque can also be identified as calcified and noncalcified plaque. Noncalcified plaque is softer and more metabolically active than hard calcified plaque, and noncalcified plaque is easier to remove than calcified plaque.

3. Prosaposin

Prosaposin is a non-enzymic glycosylated protein that plays a crucial role in lysosomes and extracellular space. Prosaposin is encoded by the PSAP gene and is involved in glycosphingolipid metabolism. Prosaposin has relatively large sizes that are between 68 kDa to 73 kDa. Prosaposin is essential in lots of vital body processes, like the activation of G protein, and stimulation of neurons, and is a precursor protein of lysosomal activator proteins. Prosaposin has varied functions due to the different places it is located. Protein deficiency can lead to lipid storage disorder because of a mutation in the PSAP gene.

3.1. Molecular Mechanism

The reason prosaposin is chosen to be involved in the protein treatment is that prosaposin can regulate the formation of plaque in the arteries. The macrophage mediates the plaque formation process in the arteries, and the signaling pathway can affect the macrophage. The inhibition of the mechanistic target of rapamycin (mTOR) decreases macrophage inflammation and metabolism, and the expression of prosaposin also decreases after the inhibition [5]. mTOR helps control several cell functions, like cell division, cell survival, and binding to rapamycin. In mTOR signaling pathways, varied parts of the body are related to it, and it controls cell proliferation, autophagy, and apoptosis [6]. The mTOR and ribosomal protein S6 kinase-1 (S6K1) shows a direct relationship in the macrophages' inflammatory process. Related to macrophages, the macrophage metabolism use mTOR signaling pathway as a regulator of it. After the inhibition of mTOR signaling, macrophage inflammation decreases [7]. To verify the reason for inflammation decreasing, the Psap gene was also found to be decreased in both mTOR inhibition and S6K1 inhibition. The prosaposin protein expresses a large amount in the plaque. The decrease in the amount of Psap led to less formation of prosaposin protein in the plaque.

3.2. Existing Experiment on Prosaposin as a Treatment of Atherosclerosis

Because the prosaposin can be used as a mediator of inflammation, targeting the prosaposin can be a potential treatment for atherosclerosis. Due to the function of prosaposin, inhibiting the mTOR and S6K1 can reduce the prosaposin in the plaque and decrease the plaque formation in the blood vessel. The clinical data of prosaposin is not shown yet, but the potential of prosaposin as a protein therapeutic is displayed. A research paper published in "Science Translational Medicine", shows the possibility of prosaposin being a mediator of inflammation in atherosclerosis. In the experiment, the researchers designed to test the effectiveness of inhibition of the mTOR on macrophages. To reach this goal, the experiment used a nanobiologic called apolipoprotein A1 (APOA1) that could deliver the drug to the target cell more efficiently. The nanobiologic including the mTOR inhibitor rapamycin (mTORi-NB) and the S6K1 inhibitor PF-4708671 (S6K1i-NB) were designed to deliver to the experimental mice and observe the change in macrophages and plaques [5]. In the experiment, the mice used were Apoe^{-/-} mice that have atherosclerosis symptoms. The mice needed to be 20 weeks old, and the mice needed to be fed the Western diet for 12 weeks to inject the nanobiologic. After 12 weeks of the Western diet, the mice needed to inject four different injections, which were phosphate-

buffered saline (PBS), unloaded nanobiologics, mTORi-NB, and S6K1i-NB. During the observations, the results showed that mTORi-NB does not affect cholesterol, but the mice treated with mTORi-NB showed narrowed plaque size. To find out what led to the narrowness of plaque, the experimenters found that the plaque collagen content is not affected, but the rate of macrophages was reduced by injecting mTORi-NB and S6K1i-NB. The mice treated with mTORi-NB had a reduction in macrophage content of about 33% to 34%. The mice treated with S6K1i-NB had a reduction of 20% in macrophage content. The effectiveness of inhibiting mTOR signaling pathways is proved in the experiment, the research paper keeps diving deep into the molecular mechanisms of it. To explore the molecular mechanism, the research continued in finding the gene expression and the intramodular hub gene. The genes of mTORi-NB and S6K1i-NB both include a decreasing hub gene, Psap. Psap is the gene of prosaposin protein and Psap encodes prosaposin. In the experiment to test prosaposin's effect on regulating inflammation, the experiment used *Ldlr*^{-/-} mice instead of the *Apoe*^{-/-} mice due to their suitability [5]. Two different groups of mice either received Psap^{-/-} or Psap^{+/+} bone marrow from the Psap^{-/-} or Psap^{+/+} mice [8]. The group with Psap^{-/-} bone marrow shows an apparent reduction of plaque macrophage, which proves the validity of using prosaposin as a target protein for atherosclerosis treatment [9]. Through this research paper, prosaposin has been proven as an effective treatment for inflammation in atherosclerosis, so prosaposin would be able to work effectively as a protein treatment.

3.3. Advantages and Limitations

Even though prosaposin can be a target protein for the treatment of atherosclerosis, limitations still exist. Prosaposin as a treatment already shows its potential to function as a therapy. The treatment can have fewer side effects compared to various surgeries, and it can have higher efficacy compared to medicines. Patients who get atherosclerosis either slight or severe can all be treated with prosaposin. Furthermore, prosaposin is a protein that the human body already included, so patients who receive this therapy would not have any intolerance. However, prosaposin therapy would be hard to treat patients with urgent situations, such as heart attacks. Prosaposin therapy needs time for the protein to make changes in the body, so patients who need to be immediately treated should not consider prosaposin as a treatment. Also, more details about the clinical use of prosaposin are not shown because it's still experimental, and in future experiments, the researchers could focus on the possibilities to put prosaposin into practice and the analysis of toxicity for various patients.

4. Hydroxymethylglutaryl-CoA (HMG-CoA)

Hydroxymethylglutaryl-CoA (HMG-CoA) is a protein that functions as a precursor for cholesterol synthesis [10]. HMG-CoA is a polytopic transmembrane protein that is closely related to the cholesterol level in the blood, which is a vital factor in plaque formation. By catalyzing HMG-CoA synthase, acetyl-CoA, and acetoacetyl-CoA's condensation happens to form HMG-CoA. HMG-CoA forms an enzyme called Hydroxymethylglutaryl-CoA reductase (HMGCR) to impact the metabolic pathway, which affects the mevalonate pathway in cholesterol synthesis [11]. The mevalonate pathway is one important metabolic process in the cell that can form several vital substances for humans, such as cholesterol.

4.1. Molecular Mechanism

HMG-CoA is chosen as a solution for atherosclerosis because of its ability to affect cholesterol levels, so the change in the HMG-CoA reductase is paralleled to the change in cholesterol synthesis. Due to the function of HMG-CoA reductase in the mevalonate pathway, decreasing HMG-CoA would lead to a decrease in plaque. Therefore, an HMG-CoA reductase inhibitor, which is called a statin, is necessary for the treatment of atherosclerosis [12]. HMG-CoA reductase converts HMG-CoA to mevalonic acid by catalyzing NADPH-, and the mevalonic acid functions in the mevalonate pathway. To stop the synthesis of mevalonic acid, the HMG-CoA reductase inhibitor would block

HMG-CoA reductase to stop it from functioning. After the HMG-CoA reductase has been inhibited, the amount of low-density lipoprotein (LDL) cholesterol would decrease [12]. LDL cholesterol makes up most of the human body's cholesterol and is one of the five lipoproteins in the human body. LDL cholesterol carries out all the fat molecules around the body, which means the high level of LDL cholesterol in the bloodstream could lead to heart problems. By decreasing the amount of LDL cholesterol in the blood, the statin subsidizes plaque, inhibits platelet aggregation, improves intradermal function, and functions as an anti-inflammatory [13]. Those functions would stabilize the amount of plaque and prevent new plaque from forming.

4.2. Exists Treatment and Limitations

HMG-CoA as a protein has been explored more than prosaposin. The HMG-CoA reductase inhibitor, statin, has already been used in several medicines in the treatment of atherosclerosis. For example, a medicine called atorvastatin is a type of statin, and it can be used to decrease the production of cholesterol that can build plaques in the blood vessels. However, due to the function of HMG-CoA reductase is to moderate the cholesterol synthesis rate, using statin in the treatment of atherosclerosis can be considered a slower method. Statin cannot be used to treat the plaque already condensed in the blood vessel walls, which shows statin should be a better medicine for patients with atherosclerosis in an early stage. Several medicines contain statin already used as a precautionary medicine for patients in clinical use. Both carotid heart disease and coronary heart disease can be released by using statin.

5. Conclusion

Atherosclerosis in both the neck and heart can cause severe symptoms due to the narrowing of people's blood vessels. Prosaposin and Hydroxymethylglutaryl-CoA (HMG-CoA) are two proteins that are proven to have possibilities to treat atherosclerosis. Both prosaposin and HMG-CoA are related to the synthesis of the plaque or the formation of important substances involved in the process. Prosaposin, without much clinical evidence, shows a potential to exert as a treatment for atherosclerosis in several consecutive experiments. The experiments show that the effect of prosaposin would lead to the decrease of macrophage, and, therefore, decrease the amount of plaque in the blood vessel. HMG-CoA is explored better compared to prosaposin. HMG-CoA reductase inhibitor, statin, worked to decrease an essential component in the blood, cholesterol, by blocking HMG-CoA from functioning in the mevalonate pathway. Statin contains medicines that are already used in clinical trials, but they mostly focus on the prevention of heart diseases and stopping the condition from deteriorating.

Protein treatment for atherosclerosis would be a potential and applicable method in the future, and fewer side effects would present because the protein treatment only affects the protein already existing in the human body. Atherosclerosis is a worldwide life-threatening disease that can lead to fatal diseases like heart attack and stroke, and future treatments should not only prevent the disease and stop it from deteriorating but also resolve the disease completely, which is what the protein treatments have the possibility to reach. In future experiments, the specific factors that can directly affect prosaposin could be tested, and the appropriate amount of dose used in the clinical trial should be explored. For HMG-CoA, future experiments could focus on the other possible effect that the inhibitor can produce, and the method to lessen the plaque in the blood vessel can also be varied. Therefore, the possibility to use prosaposin and HMG-CoA as a treatment of atherosclerosis is validated and the availability to apply them in clinical use can be explored in the future.

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