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Feasibility of Lecithin: Cholesterol Acyltransferase as the Therapeutic Target of Atherosclerosis

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Abstract:

Atherosclerosis is a global concern in this century. It is a progressive disease caused by the accumulation of cholesterol plaque, which thickens and hardens the arterial walls. It was found that low-density lipoprotein is the major leading factor because it delivers cholesterol towards the arterial walls. By contrast, high-density lipoprotein has anti-atherogenic properties due to its ability to remove cholesterol for hepatic excretion. Lecithin: cholesterol acyltransferase is an endogenous enzyme that boosts high-density lipoprotein-mediated reverse cholesterol transport. It has thereby been studied as a therapeutic target of atherosclerosis for decades. Currently, different lecithin: cholesterol acyltransferase-based therapies have different progress under development. This review illustrated the structural, biochemical, and functional properties of this enzyme. The basic rationale for two main types of this enzyme-based therapy, enzyme replacement and gene therapy, was also explained. Enzyme replacement involves artificial recombinant human lecithin: cholesterol acyltransferase. Gene therapy utilises adeno-associated viral vectors to allow enzyme expression in vivo. In addition, the advantages and limitations of each treatment were also evaluated by summarising clinical and pre-clinical data. Although whether reverse cholesterol transport is the route by which lecithin: cholesterol acyltransferase achieves its anti-atherogenic effects is not clear yet, and the variety of safety issues of the techniques, this enzyme is still a promising therapeutic target for further pre-clinical and clinical efforts.

Keywords: Atherosclerosis; cholesterol acyltransferase; high-density lipoprotein; low-density lipoprotein.

1. Introduction

Atherosclerosis affects about 75% of people aged over 60 to some degree [1]. It is categorised by the buildup of fatty plaques in arterial walls. The consequent progressive thickening and hardening of arterial walls restrict blood flow, but symptoms may not present until lethal complications like stroke and coronary artery disease are followed by heart attack [2].

The low-density lipoprotein (LDL) in the bloodstream is the major mediator of this multi-factorial process. It is one of the major forms of cholesterol that is transported in the bloodstream and extracellular fluid. In physiological conditions, LDL provides an appropriate amount of cholesterol for cells such as endothelial cells, in order to maintain normal cellular metabolism. However, when the LDL levels exceed cellular needs, they will remain in the arterial intima. The vascular cells oxidize LDL, and the oxidized LDL (ox-LDL) triggers circulating monocytes to differentiate into macrophages. The macrophages engulf these ox-LDL to become foam cells. The accumulation of foam cells forms a plaque in the arterial wall, which narrows the lumen and thereby restricts blood flow. Potential high blood pressure may break the plaque and lead to local blood clotting, which may completely clog the bloodstream to cause stroke or heart attack [2].

On the other hand, high-density lipoprotein (HDL) has a variety of anti-atherogenic functions. Similar to LDL, HDL is also a major form of cholesterol transportation. However, instead of delivering cholesterol to the endothelium and the intima, HDL achieves reverse cholesterol transport (RCT). HDL collects cholesterol from the foam cells in the intima and returns it to the liver for excretion. In addition, HDL inhibits LDL oxidation, suppressing monocyte recruitment, and inflammatory and thrombotic responses. It also promotes endothelial repair. Together, these allow the regression of the atherotic plaque and lesion [3, 4].

Lecithin: cholesterol acyltransferase (LCAT) can mediate RCT, by promoting macrophage cholesterol efflux and HDL maturation. Therefore, it has been studied for decades as a potential therapy to prevent and recover atherosclerosis [4]. The most promising LCAT-based therapies under development include enzyme replacement with recombinant human LCAT (rhLCAT) injections, and gene therapy such as adeno-associated viruses (AAV) vector-delivered human LCAT (hLCAT) *in-vivo* expression. These therapies on the market have mostly shown promising results, suggesting the potential effect of increasing RCT and reducing atherosclerosis [5]. This review intends to outline these different LCAT-based therapies, evaluating their feasibility by summarizing the experimental results. For a better understanding of the rationale and production method of these therapies, the structural and biomolecular properties of LCAT, as well as its mechanism to achieve atherosclerosis regression will also be illustrated.

2. Structural and Biomolecular Properties of LCAT

Human LCAT is predominantly produced in hepatic cells and excreted into the circulation. A minor proportion is produced in the brain and testes. The gene of human LCAT is located at the q22.1 band of chromosome 16. Its sequence size is 4.5 kb, among which the coding sequence is 1.5 kb long, with six exons and five introns containing in total of 4.2 kg bases. The mature LCAT contains 416 amino acids, where a 24-residue signal peptide from a 440 residue precursor was cleaved. LCAT is a type of α/β hydrolase fold family protein. Its secondary structure consists of four α-helices, and seven β-strands connected in the form of loops. The tertiary structure of LCAT is maintained by two disulphide bonds, between Cys50–Cys74 and between Cys313–Cys356. The Cys50–Cys74 disulfide bond allows the binding of LCAT onto the lipoprotein surface [6]. It is also essential for the dynamic LID element, which allows the conformational stabilisation of LCAT and esterification reaction. The dynamic LID element protects the active site from exposure to soluble substrates and opens when the enzyme binds to the lipoprotein surface to initiate esterification. Another role of this LID element is to selectively control the binding of lipid substrates [6, 7]. The fully processed mature has a non-glycosylated molecular weight of approximately 47 kDa and a glycosylated molecular weight of approximately 58 kDa. In addition, its extra molecular weight comes from the heavy N-linked and O-inked glycosylation. The majority of the glycosylation has important roles in both the function and the structural stability of LCAT [8]. LCAT is more hydrophilic than integral membrane proteins, but more hydrophobic than lipoproteins in plasma. Therefore, it tends to make an aggregation in the core of lipids [6].

3. Literature References

LCAT is currently the only enzyme found that can esterify cholesterol in plasma, in which two successive reactions are involved. Phosphatidylcholine (lecithin) is the main content of discoidal immature pre-ß-HDL. Upon binding onto the lipoprotein of the immature HDL, LCAT cleaves the sn-2 fatty acid from phosphatidylcholine (lecithin) and transfers it onto Ser181 of LCAT. Then, via transcacylation reaction, the fatty acid is transferred from LCAT to

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the free 3-β-hydroxyl group on the A-ring of plasma cholesterol coming from the foam cells. These steps eventually form a large proportion of lysolecithin in the HDL and cholesteryl ester [6].

LCAT promotes the RCT in multiple parallel ways. After the cholesterol is exported from the foam cells by transporters ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette subfamily G member 1 (ABCG1), the LCAT-mediated esterification of the extracellular cholesterol helps to maintain the capacity of HDL binding additional cholesterol. Therefore, a gradient of cholesterol between HDL and foam cells helps to push the cholesterol efflux, the first rate-limiting step of the RCT. In addition, cholesteryl ester is more hydrophobic than unesterified cholesterol, allowing easier access to the hydrophobic core of immature HDL. Therefore, it also provides an obstacle for the cholesteryl ester to escape from the HDL and get back to the arterial wall. Lastly, this entering of cholesteryl ester is vital for the maturation of pre-ß-HDL into spherical mature α-migrating HDL (α-HDL). The mature α-HDL then circulates to the liver for RCT, transferring its cholesterol and cholesteryl ester content to hepatic cells, either directly via scavenger receptor type BI (SR-B1) receptors or indirectly by cholesteryl ester transfer protein (CETP). As a result, these cholesterol and cholesteryl esters are eventually excreted via bile [5, 6].

4. Enzyme Replacement: rhLCAT Protein Administration

rhLCAT is an artificial LCAT that is intravenously administered to mimic endogenous LCAT enzyme function. It is the most promising approach in raising LCAT activity, with the progress of the phase II clinical trial recently [5]. Among a number of different rhLCATs on the market, APC-501 and MEDI6012 have shown the most significant anti-atherogenic effect [9-12].

4.1 General Production Method

The general production method of rhLCATs firstly involves isolation of human LCAT cDNA from human samples using sequence-specific restriction enzymes and polymerase chain reaction (PCR). The targeted gene is then inserted into a pre-designed vector plasmid in vitro by restriction enzymes and ligases, followed by bacterial amplification of the recombinant plasmid. Due to the vital structural and functional role of the post-translational modifications of LCAT including the N- and O-glycosylation, transdusing bacteria for the production of this enzyme cannot fully mimic its endogenous function. Therefore, instead, the mammalian cell line is transfected with the recombinant plasmid to produce rhLCAT. The rhLCAT was then purified and formulated for intravenous injection into either human or mammalian models. In specific, for the establishment of ACP-501, the Chinese hamster ovary cell line was used as the mammalian source of production [10]. MEDI6012 is an identical enzyme to APC-501, with a higher purification and increased specific enzymatic activity [11].

4.2 Clinical Trial Results

In the clinical trials of APC-501 injection into coronary artery disease patients who have low HDL-cholesterol (HDL-C), Shamburek et al. found a dose-proportional increase of cholesteryl ester and HDL-C. The decreased immature pre-ß-HDL and increased mature HDL suggest promoted cholesterol excretion. In addition, they have also tested the safety and tolerability of this drug, pushing APC-501 into further clinical trials [9]. The clinical trials in familial lecithin: cholesterol acyltransferase deficiency patients have suggested similar results, where LDL-cholesterol (LDL-C) was increased more slowly than HDL-C. Also, an increased cholesteryl excretion concluded from immature pre-ß-HDL reduction accompanied by mature α-HDL augmentation [10].

MEDI6012 was also administrated to stable coronary heart disease patients in clinical trials, where these patients have reduced LCAT and HDL activity. Richard et al. have similarly found a dose-dependent increase of HDL-C and a decrease of LDL-C, suggesting increased cholesteryl excretion. They have also suggested the adverse effect of MEDI6012 did not significantly differ from placebo. Notably, this drug repaired the defective HDL function by promoting non-ABCA1 cholesterol efflux capacity. The repaired HDL therefore allows endothelial protection against atherosclerosis [11]. In the clinical and in-vitro study of George et al. with acute coronary syndrome, they suggested an increased HDL activity, and the resulting restoration of HDL-mediated endothelial NO production. NO maintains blood pressure and vascular tone to protect the vessels. In addition, they have found that CETP, the indirect way of cholesterol excretion, was increased [12].

4.3 Limitations and Improvements

Although these promising rhLCATs have shown increased mature HDL particles to suggest promoted cholesterol excretion, most of the conclusion was only based on analysis of HDL, LDL, and cholesterol particles [9-12]. The downstream RCT was actually not assessed in most of the trials, which raised the possibility that these HDL-raising drugs may have lipid metabolism activity different from the physiological activity of endogenous LCAT.

Therefore, a more direct assessment of RCT is required, in order to explore the hypothesised positively correlated connection between the rise of HDL and the promotion of RCT. [5] In addition, most of these clinical trials focused on atherosclerotic patients with a significant reduction in endogenous HDL activity [9, 10, 11, 12]. Therefore, the possibility of applying rhLCAT administration to treat atherosclerosis in patients with normal HDL activity is yet to be investigated.

5. Gene Therapy: Adeno-Associated Viral Expression

Gene therapy of LCAT was first applied in 1996, where adenovirus was selected as the vector to allow the in-vivo expression of LCAT in a mouse model. However, the use of adenovirus in general was found to have severe adverse effects in clinical trials focusing on other genes, such as inflammatory responses and multi-organ failure. Therefore, lentiviral vector and adeno-associated viral (AAV) vector were developed to express LCAT, instead [5]. Because lentivirus tends to integrate into the host genome in vivo, its cancer-prone feature was found in some clinical trials focusing on other genes [13]. By contrast, the AAV vector is now the most ideal type of vector under development, due to its specific tropism, non-pathogenicity, low immunogenicity, and cost-effectiveness of production. There are more than 100 AVV vector serotypes identified currently, each having a distinct capsid structure and tropism [14]. AAV8 has its unique tropism targeting the liver, which is the endogenous site of LCAT production. It has shown the most promising pre-clinical trial results among all serotypes [15].

5.1 General Production Method

The production of AAV8-hLCAT involves the following general principles. Because AAV vectors have a small packaging capacity of gene sequences shorter than 5kb, in the selection of hLCAT gene sequence, the shorter cDNA rather than genome DNA is normally utilised. The cDNA of hLCAT is isolated from a human cell line and then cloned by PCR. In order to further boost the liver tropism of AAV8, the hLCAT cDNA should then be inserted downstream of a liver-specific promoter within a plasmid construct. In addition, this plasmid construct would include a specific insertion to reduce the immunogenicity of this entire AVV8 product in vivo and stabilise gene transfer. Reporter genes may also be inserted to localize the infection and monitor hLCAT expression. Helper-free triple plasmid transfection is a classical method to establish viral vectors like AAV8, where an adenoviral helper plasmid, a packaging plasmid, and a pAAV cis plasmid are normally used to co-transfect mammalian cells, to provide a large amount of AAV8 construct for intravenous administration [16].

5.2 Pre-Clinical Trial Results

Until now, AAV8 has only been assessed in pre-clinical trials. In FLD patient-like mice models, the effect of AAV8-mediated hLCAT gene delivery in vivo was analysed by a detailed plasma lipid profile investigation. The result suggested a significant increase in HDL cholesterol, HDL maturation in terms of particle size, and HDL cholesteryl esters. The reduction in LDL cholesterol and plasma triglycerides was also found. These together had similar effects as rhLCAT therapy [16]. Guo et al. conducted another pre-clinical trial has applied a similar FLD patient-like LCAT homozygous knockout hamster model to investigate AAV8-hLCAT. Similarly, they found a significantly improved atherogenic lipoprotein profile. In detail, plasma HDL-C was increased upon the AAV8 construct. Also, the reduction in plasma free cholesterol concentration relative to total plasma cholesterol concentration, and the reduction in plasma triglycerides was found. Notably, they have also directly quantified the severity of atherosclerosis in these hamsters in terms of area of lesion in different parts of the artery [17]. These suggested that AAV8-hLCAT have the potential to treat human atherosclerosis.

5.3 Limitations and Improvements

Despite being the safest viral vector to deliver hLCAT genes, AAV8-hLCAT still show genotoxicity to some degree. Also, its immunogenicity in the host, gene transfer efficiency, as well as time and cost of production may vary largely depending on multiple factors. Therefore, consistent effort on this technique is required to achieve optimisation, in order to push this technique into clinical trials [5].

In addition, an alternative technique, ex vitro gene therapy, may minimise most of these side effects and limitations mentioned above, especially immunogenicity. Among a variety of human cell types in vivo, genetically modified adipocytes are the most suitable choice to deliver hLCAT back to the same individual. One of the reasons is the well-established experience in plastic surgery and reconstructive surgery, where aspirated adipocytes are often used for autologous tissue transplantation and are considered with minimal risk. Also, because adipose tissue is a well-vascularised endocrine and secretory organ, it is capable of raising a therapeutic systematic level of hLCAT. Besides, the annual tissue renewal rate as low as

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10% allows a prolonged correction of the hLCAT level of approximately 10 years [18]. However, these cannot simply conclude the newly developed ex vivo gene therapy is comprehensively better than in vivo gene therapy. Firstly, AAV8 in vivo gene therapy allow tropism towards the liver, the natural site of production of LCAT. Instead, adipose tissue does not produce a significant amount of LCAT endogenously. These may lead to differences in post-translational modifications of LCAT, which may have unpredicted side effects. Also, the ex vivo transfection process of adipocytes may induce oncogenic transformation that is hard to detect until years after transplantation. The low cell survival rate after transformation should also be solved [5].

6. Conclusion

Atherosclerosis has been a global healthcare issue, where LDL is the driving factor and HDL is the rescue factor. LCAT is found to improve HDL maturation and the downstream RCT, which leads to atherosclerosis regression physiologically. Therefore, LCAT is been studied for decades as a therapeutic target to treat atherosclerosis. In this review, the structural and biochemical properties of LCAT, as well as the mechanism of its anti-atherogenic activity are illustrated, in order to provide a basic rationale for the understanding of LCAT-based treatments. Enzyme replacement with rhLCAT and gene therapy with AAV8, the two most promising types of LCAT-based treatment, were explained and evaluated.

The rhLCAT is currently the only LCAT-based therapy entered clinical trials. It is a direct intravenous injection of rhLCAT following in vitro production of this enzyme in mammalian cell lines. Two types of rhLCAT, ACP-501 and MEDI6012, have been shown to improve the atherogenic lipid profile significantly in patients with LCAT deficiencies. Also, they can lead to increased cholesterol excretion. However, no direct text on RCT was conducted clinically, which remains the possibility that these HDL-rasing drugs may not have the same route as endogenous LCAT to achieve cholesterol excretion. Also, all of these clinical trials aimed to correct the preexisting low-LCAT level to a physiological level and to treat low-LCAT-related atherosclerosis. This raised the need to investigate the effect of rhLCAT in atherosclerotic patients with normal LCAT levels.

The AAV8 vector is currently only applied to pre-clinical animal models. It is a virus-delivered hLCAT gene expression in vivo, especially in the liver, which is the natural site of endogenous LCAT production. The pre-clinical results were similar to the enzyme replacement therapy of rhLCAT, where an improved atherogenic lipid profile was also found in transgenic mice model simulating LCAT deficiencies. Notably, the quantification of the atherotic lesion area in different parts of the arteries directly suggested the antiatherogenic effect of AAV8-hLCAT. However, optimisation for AAV treatment is required, in order to solve restrictions such as genotoxicity, immunogenicity, gene transfer efficacy, and cost-effectiveness. Another newly established alternative version of AAV8-based in vivo gene therapy is the ex vivo gene therapy using autologous adipocytes. This ex vivo transgenic adipocyte transplantation minimises the immunogenicity of direct AAV injection. Nonetheless, adipocyte is not the natural site of production of LCAT and may be oncogenic after in vitro editing .

Overall, LCAT is one of the most promising protein targets to treat atherosclerosis. Further effort is required to optimise the safety and efficiency of AAV-based therapies. Also, the metabolic process of therapeutic LCAT in RCT should be investigated.

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