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Lipoprotein retention in the vascular wall – a potential therapeutic target?

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Accumulation of lipoproteins in the vascular wall is one of the main causes of atherosclerosis. Inhibiting this type of binding could potentially limit the accumulation of lipoproteins in the vascular wall.

Cardiovascular disease is the leading cause of death worldwide (1). Extensive research is ongoing into the accumulation of lipid in plaques, activation of immune cells and the role of inflammation in the development of cardiovascular disease. The importance of the extracellular matrix, by contrast, has received little attention.

The incidence of cardiovascular disease has been declining in Norway since the early 1970s (2). Improved medical treatment as well as reductions in smoking and in the intake of trans and saturated fats have been key factors in this development (3). Atherosclerosis is the underlying cause of 80–85 % of all cardiovascular disease (3). This imperceptible process is therefore of great public health importance. Its development occurs over an extended period, and organ manifestation is typically seen after the age of 50 years, and somewhat later in women than in men (4).

The development of atherosclerosis is the result of a number of factors, several of which are targeted by the current standard treatments for patients in various at-risk groups. Elevated cholesterol levels, hypertension and hyperglycaemia are key risk factors that can be treated effectively with drugs and with dietary and lifestyle interventions. Plaque formation is primarily seen in large and medium-sized arteries, with the most important sites of accumulation being the aorta, coronary arteries and precerebral arteries (Box 1) (5, 6).

Box 1 Content of atherosclerotic plaques (6)

Lipid deposits
Accumulations of immune cells
Increased number of smooth muscle cells
Increased amount of extracellular matrix

Extracellular matrix in the vascular wall

In normal arteries, much of the extracellular matrix (up to 50 %) consists of elastic fibre proteins, collagen and smaller amounts of proteoglycans (6). As lesions develop in the vascular wall, the ratio of these components gradually changes. In the early stages, the amount of proteoglycans increases sharply, while elastic fibres and collagen become less abundant. In the later stages, however, the amount of collagen increases, while elastic fibres and proteoglycans are reduced. Matrix components are also found in the glycocalyx layer (7), which coats the surface of the vascular wall facing the bloodstream. The role of the glycocalyx in the development of atherosclerosis has been little studied to date.

The various components of the extracellular matrix have different structures, properties and functions in the vascular wall. Changes in their relative abundance will affect the functions of the individual components and of the vascular wall in general, especially in vulnerable areas such as the aorta, coronary vessels and cerebral arteries. The thickening of the intimal layer of the vascular wall results both from an increase in the number of smooth muscle cells as well as an increase in the extracellular matrix produced by those cells. A key factor contributing to the increased vessel stiffness (6) seen with more advanced atherosclerosis is the accumulation of extracellular matrix and of cross-links between matrix components. These cross-links make the matrix more resistant to enzymes that are usually responsible for the dynamic turnover of connective tissue in vascular walls (8).

Atherosclerosis is the underlying cause of 80–85 % of all cardiovascular disease

Proteoglycans are a key component of the extracellular matrix in the vascular wall and are of great importance for the development of atherosclerosis. Proteoglycans are proteins with highly negatively charged polysaccharide chains known as glycosaminoglycans (9). These chains contribute to the binding of water that generates the hydrated and flexible matrix structures seen in tissues exposed to high pressures, such as in arterial trees and in cartilage, including in knee joints. In addition, proteoglycans bind low density lipoprotein (LDL) and very low density lipoprotein (VLDL) as well as a number of proteins and apoproteins that are important in lipoprotein turnover and function, as shown in Table 1. The strongest binding is usually seen with heparan sulphate, primarily due to its high charge density, but also because of specific structures in its glycosaminoglycan chains. In the case of apolipoprotein B (apoB), a defined part of the protein binds to glycosaminoglycans, namely regions that are exposed on the surface of the protein and that contain short sequences of basic and positively charged amino acids. Proteoglycans are therefore important in normal lipoprotein turnover, in part through binding lipoproteins via apoB or apoE in association with lipoprotein receptors (10).

Table 1

Proteoglycans and lipoproteins

Ligand	Function
Lipoprotein lipase	Binds to heparan sulphate on endothelial cells
Hepatic lipase	Binds to heparan sulphate in hepatocytes

Ligand	Function
ApoB	Binds to heparan sulphate. Acts in association with the LDL-receptor
LDL and VLDL	Bind to heparan sulphate and chondroitin/dermatan sulphate
Antithrombin	Binds to heparin and heparan sulphate

The quantity of proteoglycans increases upon development of atherosclerosis, especially in the initial phase. Through the use of advanced extraction methods and structural studies, it has been possible to identify the proteoglycans that accumulate in atherosclerotic plaques. The small leucine-rich proteoglycans biglycan and decorin (11) have received much attention because they bind to collagens. Versican and perlecan are other important proteoglycans implicated in these processes.

Relevance of proteoglycan to new treatments

The role of proteoglycans in fibrosis development in atheromas is well documented, particularly for the extracellular proteoglycans described above. For cell surface proteoglycans, recent studies have revealed important functions relevant to the current treatment of patients with familial hypercholesterolaemia, or with diabetes, overweight and other cardiovascular risk diagnoses (12).

Statins and cholesterol absorption inhibitors such as ezetimibe are standard treatments for lowering cholesterol levels in these patient groups. In addition, a treatment has been developed that inhibits proprotein convertase subtilisin/kexin type 9 (PCSK9). This is an enzyme with the ability to bind the LDL receptor (LDLR) in the liver. The LDL/LDLR/ PCSK9 complex is taken up by liver cells and further internalised by hepatocytes, whereupon the complex dissociates and the LDLR returns to the cell surface. Here it can take up new LDL particles from the circulation. PSCK9 targets LDLR towards lysosomes for degradation, resulting in lower surface levels of LDLR and thus higher LDL levels in the blood. Antibody-based drugs are now available that can bind PCSK9 and thereby improve the recirculation of LDLR, reducing both circulating LDL cholesterol and the incidence of cardiovascular disease (13, 14).

Individuals with type 1 or type 2 diabetes could conceivably benefit from this form of prophylactic treatment

Recent data show that in hepatocytes, surface proteoglycans with heparan sulphate chains bind PCSK9 and then transfer it to the LDLR. The binding sites on both PCSK9 and heparan sulphate have now been identified. Work is underway to develop heparan sulphate-like substances that can interfere with the PCSK9–LDLR interaction. The goal is to reduce plasma LDL levels to the same degree as the antibody-based drugs that have been approved for clinical use.

Lipoprotein retention in the vascular wall

Work is also underway to develop drugs that act on the processes leading to the accumulation of LDL in vascular walls. Under normal physiological conditions, there will be a dynamic equilibrium between the concentrations of lipoproteins in the circulation and the interstitium. During the formation of atherosclerotic plaques, levels of extracellular proteoglycans increase in the intima layer of vessels. In individuals with elevated levels of lipoproteins in the blood, especially apoB-containing lipoproteins such as LDL, this will increase the retention of lipoproteins in the vascular wall. The tightly packed negative charges in the glycosaminoglycan chains of versican, perlecan, decorin and biglycan will increase the binding of lipoproteins to the matrix in the vascular wall. This is the basis of the so-called 'response-to-retention' hypothesis (15). The essence of this hypothesis, which has strong support from advanced animal studies, is that lipoproteins – through basic and positively charged regions (in apoB, for example) – bind to proteoglycans in the extracellular matrix. This may lead to the formation of complexes that are then taken up by activated macrophages in the vascular wall, or to the deposition of

lipoprotein–proteoglycan complexes in the matrix. Both will contribute to atheroma formation with lipid and matrix accumulations as key components, in addition to an increased influx of immune cells such as macrophages.

Lipoprotein retention in the intima and media

Studies have been conducted to determine which proteoglycans bind LDL in the intima, and have shown biglycan to be associated with lipoproteins. In plaques, lipid accumulation is also seen in macrophages that have converted to foam cells, as well as extracellularly, including in association with biglycan. A number of experimental and animal studies have attempted to interfere with the binding of LDL to biglycan. Endostatin is a fragment from the basement membrane proteoglycan collagen XVIII, which acts as an inhibitor of angiogenesis and which has been tested in oncotherapy. Endostatin has also been shown to reduce atherosclerosis in animal studies (16), as has an antibody raised against glycosaminoglycan chains (17). Both of these angles of attack aim to inhibit early lipoprotein retention, thereby helping to slow the development of atherosclerosis.

The interaction between LDL and biglycan is well documented, but significantly more detailed studies of the binding between these two components will be required in order to develop a treatment that could be tested in clinical trials. Studies with well-established and validated systems for measuring binding are already in progress to clarify in detail which parts of biglycan and of apoB in LDL are responsible for the binding. Inhibition studies with endostatin and defined fragments of apoB are also undergoing validation. This will make it possible to test libraries of substances to identify those that can inhibit binding. This approach is also used in drug development. The system is well validated, but resource intensive.

It may also be possible to approach the problem from the opposite direction by identifying substances that can be tested in biological systems in which labelled LDL is exposed to human vascular tissue isolated by surgeons, for example in connection with heart transplants. Bioreactor systems for this type of testing are commercially available and well-validated. If we can inhibit the binding of LDL to human vascular tissue with defined substances in a library, we will have come a long way towards possible clinical trials. The long-term goal must be to offer prophylactic treatment that inhibits atheroma formation, especially in at-risk groups.

Potential practical significance

Studies have shown that proteoglycans are involved in the early stages of atherosclerosis, in which these highly negatively charged macromolecules affect both the expansion and retention of lipoproteins in the matrix. If a drug based on reducing the binding of LDL to the extracellular matrix in the intima is to be used, we must consider which patient populations to target. Individuals with type 1 or type 2 diabetes could conceivably benefit from this type of prophylactic treatment because of their increased risk of cardiovascular disease even without high LDL. Whether individuals with familial hyperlipidaemia are suitable for treatment will depend on their age and their disease severity. Patients with rheumatic diseases may also stand to benefit.

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