The pathogenesis of atherosclerosis

Michael Mahmoudi

Abstract

Cardiovascular disease remains the world's leading cause of morbidity and mortality. The myriad of environmental risk factors and predisposing genes leads to a complex multifactorial disease process characterized by a fibro-proliferative and inflammatory process. This results in an activation of cytokines, growth factors and vasoregulatory mechanisms, and leads to intimal thickening and ultimately luminal obstruction. Progressive luminal obstruction or acute rupture of the atherosclerotic lesion can lead to devastating complications such as myocardial infarction, stroke and death.

Keywords Atherogenesis; endothelial dysfunction; inflammation; MRCP; reactive oxygen species; statins

Key points

- Atherogenesis is a chronic inflammatory disorder characterized by endothelial dysfunction and the accumulation of a number of cellular components within the vascular intima
- Atherogenesis is enhanced by a number of risk factors, including hypercholesterolaemia, smoking, diabetes mellitus, hypertension and family history
- Atherogenesis can be modulated by statins

The pathogenesis of atherosclerosis

Atherosclerosis is a disease process characterized by the interaction between endothelial dysfunction, subendothelial inflammation and the 'wound healing response' of vascular smooth muscle cells (VSMCs). The atherosclerotic plaque is characterized by the accumulation of lipids, inflammatory cells, VSMCs and connective tissue within the arterial intima. The major cellular components of the atherosclerotic plaque are: VSMCs and lymphocytes, most abundant in the fibrous cap; macrophages, which dominate the lipid core; and platelets (Table 1).

Atherogenesis should be viewed as a dynamic continuum of several stages, involving endothelial activation, lipid entry and modification, VSMC migration and persistent inflammation that ultimately leads to the formation of an atherosclerotic plaque (Figure 1).

Endothelial dysfunction

Vascular endothelial cells maintain the balance between vasodilatation and vasoconstriction, inhibition and stimulation of VSMC proliferation and migration, and thrombosis and fibrinolysis.[1] Endothelial dysfunction is recognized as one of the earliest precursors of atherogenesis and describes an imbalance between endothelial-dependent vasodilatation and endothelium-dependent contraction. One of the hallmarks of endothelial dysfunction is a loss of normal endothelial-dependent vasodilatation.

Vasodilatation is predominantly achieved by nitric oxide (NO), as well as prostacyclin, bradykinin and endothelium-derived hyperpolarizing factors. NO, which is synthesized from L-arginine by the enzyme NO synthase and requires tetrahydrobiopterin as a co-factor, mediates its downstream function through the cyclic guanosine monophosphate second messenger system. In addition to its vasodilatory action, NO mediates the inhibition of leucocyte adhesion to endothelial cells, maintenance of VSMCs in a non-proliferative state, and inhibition of platelet aggregation.

Vasoconstriction is mediated predominantly by endothelin 1 and angiotensin II. Endothelin 1 also potentiates the action of other vasoconstrictors such as angiotensin II, noradrenaline (norepinephrine) and serotonin, as well as participating in leucocyte and platelet activation.

Numerous studies have demonstrated an association between conventional risk factors for atherosclerosis, such as hypertension, smoking, diabetes mellitus and hypercholesterolaemia, and endothelial dysfunction. There is also evidence supporting increased vascular production of superoxide species that react rapidly with NO leading to the production of peroxynitrite anions and loss of bioactivity of NO. The generation of such highly reactive oxygen species (ROS) promotes the oxidative degradation of tetrahydrobiopterin, leading to decreased production of NO.

Maintenance of inflammation and plaque progression

The decreased bioavailability of NO is accompanied by the expression of a spectrum of endothelial cell surface receptors such as vascular cell adhesion molecule 1, intercellular adhesion molecule 1 and P-selectin, which bind platelets, monocytes and T lymphocytes. This initiates an inflammatory process that ultimately leads to the formation of an atherosclerotic plaque. The migration of these cells into the subendothelial space is facilitated by chemo-attractants such as monocyte chemoattractant protein 1, oxidized low-density lipoprotein (LDL), macrophage colony-stimulating factor and a distinct family of T cell chemo-attractants.

Platelets are one of the first cell types to arrive at sites of endothelial activation. The platelet surface receptors Ib and IIb/IIIa interact with ligands on the surface of endothelial cells and contribute to endothelial activation. Inhibition of such interaction in hypercholesterolaemic mice has been shown to reduce leucocyte infiltration and progression of atherosclerosis.

The entry of monocytes into the subendothelial space and their subsequent transformation into macrophages is a key step

Michael Mahmoudi *BSc MB BS PhD FACC FRCP is a Consultant Cardiologist and Honorary Associate Professor of Interventional Cardiology at the University Hospital Southampton NHS FT, UK.*
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Major cells implicated in atherogenesis

Key cells in atherosclerosis	Physiological role	Pathological role
Endothelial cells	Maintaining vascular tone, maintaining haemostasis	Endothelial dysfunction is a key first step in atherogenesis
Vascular smooth muscle cells	Maintaining vascular contractility	Formation of the fibrous cap; reduced numbers in regions of plaque rupture
Lymphocytes	Innate and adaptive immunity	Secretion of cytokines that contribute to the inflammatory response
Monocytes/macrophages	Innate and adaptive immunity	Formation of foam cells; contribute to the formation of the necrotic core
Platelets	Vascular thrombosis at sites of injury	Contribute to endothelial activation; key role in thrombosis acute coronary syndrome

Table 1

in atherogenesis. Once in the subendothelial space, the macrophages express the necessary scavenger receptors, for example SR-A, CD-36 and LOX-1, to internalize modified lipoproteins. Internalization of these particles gives rise to lipid-laden macrophages, so-called foam cells. The macrophages also produce cytokines such as interleukin-1, tumour necrosis factor-α, transforming growth factor-β, proteolytic enzymes and growth factors such as insulin-like growth factor 1. These are critical in

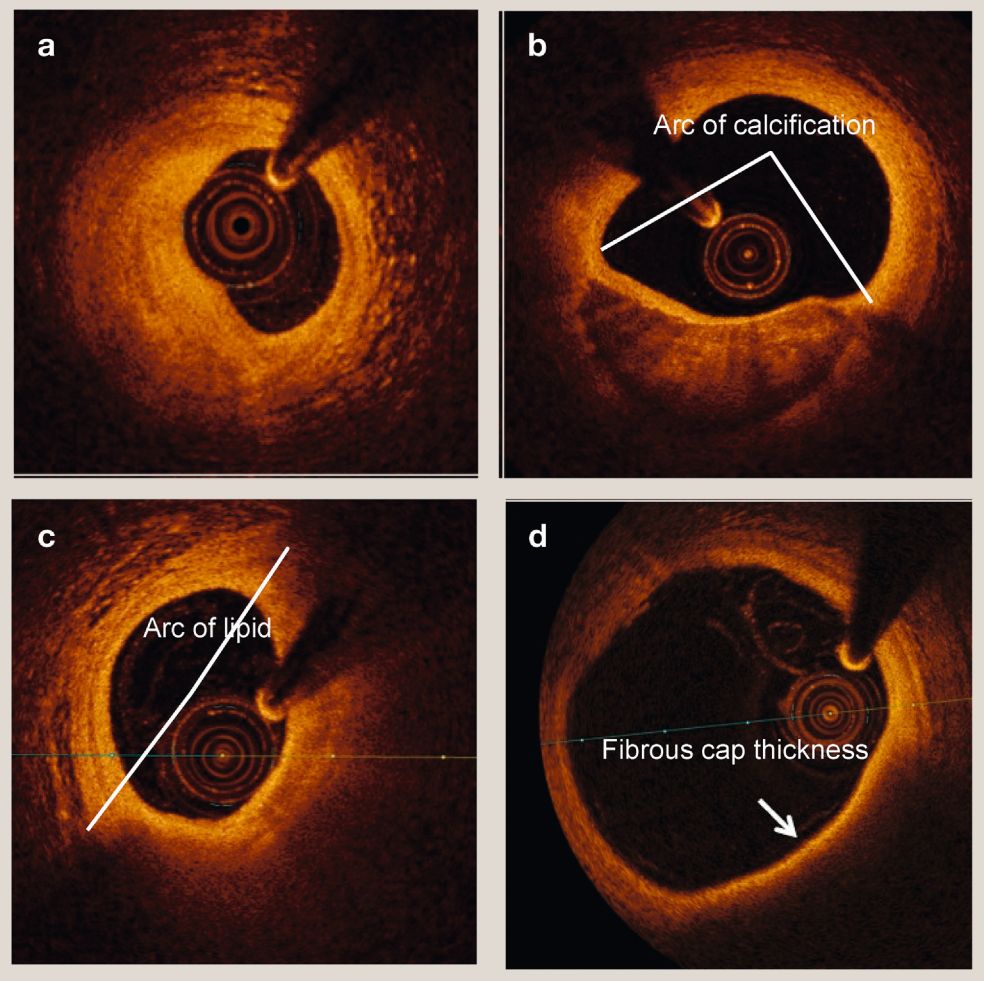

Figure 1 Examples of coronary atherosclerotic plaques. The plaques have been visualized by frequency domain optical coherent tomography: (**a**) fibrotic, (**b**) fibrocalcific, (**c**) lipid-rich, and (**d**) thin cap fibroatheromatous.

maintaining a chemotactic stimulus for adherent leucocytes, enhancing the expression of scavenger receptors and promoting macrophage replication.[2]

The entry of lymphocytes into the intima is mediated by vascular cell adhesion molecule 1. Once in the intima, T lymphocytes are activated in response to antigens such as oxidized LDL and begin to secrete a spectrum of cytokines that can interact with macrophages. CD40/CD40L interaction between the activated T lymphocytes and macrophages results in the production of tissue factor, matrix metalloproteinases and other cytokines that enhance the inflammatory response. In the presence of persistent endothelial activation and inflammation, the atherosclerotic plaque progresses from a fatty streak to a more complex lesion.[3]

Medial VSMCs are predominantly responsible for maintaining vascular tone, which is primarily maintained by matrix proteins and surface integrins. The inflammatory environment created within the plaque provides the stimulus for VSMCs to migrate to the intima. On migration, VSMCs undergo a phenotypic change characterized by a reduction in the content of contractile proteins and an increase in synthetic organelles. They produce proteinases that facilitate their migration, growth factors facilitating their proliferation, and matrix proteins such as collagen and elastin, leading to the formation of the fibrous cap.

There is continuing debate over the origin of intimal VSMCs, with some evidence to suggest that intimal VSMCs arise from circulating progenitor cells. For example, studies on sex-mismatched bone marrow transplant recipients have shown that VSMCs in the atherosclerotic lesions of bone marrow recipients can derive from the donor bone marrow. Furthermore, cells expressing VSMC-specific markers have been isolated from human peripheral blood mononuclear cells *in vitro*, but the contribution and signal for recruitment of these cells to the intima remain to be elucidated.

Reactive oxygen species

Cellular respiration leads to the generation of partially reduced oxygen species called ROS. The major enzymatic sources of ROS are reduced nicotinamide adenine dinucleotide phosphate oxidase, xanthine oxidase, NO synthase, myeloperoxidase and lipoxygenases.

Under normal physiological conditions, ROS serve as important components of cellular signalling pathways. A balanced redox state is established between the major ROS-producing systems and the major antioxidant systems. The latter includes catalase, α-tocopherol, ascorbic acid, superoxide dismutase, glutathione peroxidase and glutathione *S*-transferase, which conjugates reduced glutathione to hydrophobic organic compounds and glutathione.

Excess production or reduced degradation of ROS by the antioxidant defence system imposes an oxidative burden on the cellular environment, leading to the modification of many biomolecules and functional defects. Elevated concentrations of oxidatively modified proteins and lipid peroxidation products have been identified in patients with coronary artery disease. Epidemiological studies have also shown an association between risk factors for cardiovascular diseases and elevated ROS concentrations.

Genome-wide studies

A number of single nucleotide polymorphisms (SNPs) have been associated with atherosclerosis. The most thoroughly investigated SNP occurs within the chromosome 9p21.3 locus, which has been linked to early-onset coronary artery disease and intracerebral and aortic aneurysm formation. What has remained uncertain is the molecular mechanism that links such SNPs to atherosclerosis, particularly given that these regions are often devoid of any known protein-encoding genes.

Interestingly, the 9p21.3 locus is located adjacent to a cluster of cell-cycle regulating tumour suppressor genes such as cyclin-dependent kinase inhibitors encoding regulatory proteins, for example p14, p15 and p16, that are responsible for apoptosis, cellular proliferation and ageing. Human atherosclerotic lesions have been shown to exhibit an inverse relationship between the expression of these proteins and VSMC proliferation in the 9p21 variant, indicating a link between this SNP and susceptibility to atherosclerosis.

Statins as disease-modifying agents

The enzyme 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase is the rate-limiting enzyme in cholesterol synthesis. Statins reversibly inhibit this enzyme, leading to a reduction in hepatic synthesis and secretion of lipoproteins, as well as upregulation of LDL receptors on hepatocytes, and apolipoprotein E- and B-containing lipoproteins. Statins also inhibit the synthesis of other intermediate metabolites involved in the post-translational modification of a variety of proteins, such as the guanosine triphosphate-binding proteins Ras and Rho. Rho inhibition has been postulated to mediate some of the pleiotropic effects of statins.

A large body of evidence supports the beneficial effects of statins in both the primary and secondary prevention of coronary artery disease-related events. For example, the West of Scotland Coronary Prevention Study (WOSCOPS), a primary prevention study, demonstrated a 31% relative risk reduction for coronary events in the pravastatin group.[4] There were similar risk reductions for non-fatal myocardial infarction (31%), death from coronary heart disease (33%) and death from cardiovascular causes (32%).

The Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) study demonstrated that atorvastatin 80 mg/day produced a 34% reduction in C-reactive protein, and a 13% reduction in serum amyloid A protein, indicating that statins reduce the inflammatory response associated with acute coronary syndromes.[5] At this dose, atorvastatin has also been shown in the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) study to reduce the progression of coronary plaque growth.

Statins have also been shown to modify many of the steps involved in atherogenesis, including endothelial dysfunction (by increasing the mRNA half-life of endothelial NO synthase (eNOS), augmenting eNOS function, and attenuating endothelial cell apoptosis). This inhibits leucocyte—endothelial cell interaction and reduces neo-intimal inflammation and macrophage infiltration. ◆

KEY REFERENCES

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TEST YOURSELF

To test your knowledge based on the article you have just read, please complete the questions below. The answers can be found at the end of the issue or online here.

Question 1

A 25-year-old man was killed in a road traffic accident. At autopsy, early manifestations of atherosclerosis were noted in his aorta.

What is the first stage in the pathogenesis of an atherosclerotic plaque?

A. Decreased bioavailability of nitric oxide
B. Migration of fibroblasts into the subintimal space
C. Migration of lymphocytes into the intima
D. Platelet activation and adhesion to endothelial receptors
E. Increased bioavailability of endothelin-1

Question 2

A 65-year-old man sustained an out-of-hospital cardiac arrest. Despite cardiopulmonary resuscitation he was pronounced dead at the scene. At autopsy, there was evidence of a ruptured plaque within the lumen of the left main stem with overlying thrombus. Microscopy revealed extensive foam cell accumulation within the ruptured plaque.

What is the origin of foam cells?

A. Vascular smooth muscle cells
B. B lymphocytes
C. Fibroblasts
D. Histiocytes
E. Macrophages

Question 3

A 45-year-old woman presented with an acute myocardial infarction. She was prescribed atorvastatin 80 mg daily and was recommended to take this dose for the rest of her life if she experienced no adverse effects.

Apart from reducing serum cholesterol, what is the other main beneficial effect of atorvastatin at a tissue level?

A. Reduced inflammation
B. Stabilization of the fibrous cap
C. Stimulation of cholesterol efflux
D. Increased vascular vasodilatation
E. Reduced monocyte migration