Newer antiatherosclerosis treatment strategies

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ABSTRACT

Atherosclerosis has been a target of much clinical and molecular research. As a result of this extensive research, it is amply clear that atherogenesis is a multifactorial process involving an interplay of metabolic, immune and inflammatory mechanisms. Antiatherosclerotic strategies are today aiming for a multipronged approach targeting each arm of this multifactorial process. The newer agents under development can be divided into three broad categories: anti-inflammatory agents, modulators of intermediary metabolism and antiatherosclerosis vaccines. Potential targets for anti-inflammatory agents include inhibition of conversion of low-density lipoprotein (LDL) to oxidised LDL, blocking or downregulation of cell adhesion molecules, chemokine modulation and macrophage receptor blockade. Beyond inhibition of plaque formation, efforts are also ongoing to develop agents which stabilise the plaque by increasing its fibrous content and inhibiting its disruption. So far as research in the sphere of intermediary metabolism is concerned, the focus is now primarily on raising high-density lipoprotein and promoting reverse cholesterol transport; potential targets include cholesteryl ester transfer protein, liver X-receptor, lecithin cholesterol acyltransferase and high-density lipoprotein mimetics. Acyl-coenzymeA: cholesterol acyltransferase is another enzyme whose selective and differential inhibition is under active investigation. The concept of immunisation against a non-communicable disease such as atherosclerosis is still in its nascent stages. However, with increasing evidence to suggest the role of antigen-specific T-cell-mediated immunity in atherogenesis, this approach is potentially promising. Possible antigens under evaluation include oxidised LDL and its subparticles, heat-shock proteins and cholesteryl ester transfer protein. With cardiovascular disease being the single leading cause of death worldwide, the development of a safe and successful antiatherosclerosis strategy (possibly employing a combination of agents acting at various levels) will indeed be a major 21st-century achievement.

INTRODUCTION

Atherosclerosis has been a target of much clinical and molecular research—and with good reason, no doubt, for cardiovascular disease is today the single leading cause of death worldwide.[1] Antiatherosclerotic properties have been ascribed to oral antidiabetics (eg, thiazolidinediones), lipophilic beta-blockers, ACE inhibitors with sulfhydryl group and dihydropyridine calcium-channel blockers.[2] However, their efficacy in retarding atherosclerosis—beyond that conferred by virtue of their primary risk factor modifying action—is far from satisfactory.

As a result of extensive research, it is amply clear that what was once believed to be a simple process of cholesterol deposition in a milieu of cholesterol excess is, in fact, a multifactorial process involving interplay of metabolic, immune and inflammatory mechanisms.[3] This knowledge has brought to the fore multiple newer targets as well as newer strategies against atherosclerosis. While no single agent can be expected to eliminate cardiovascular disease, a multipronged approach, targeting each arm of this multifactorial process, is what may ultimately help us conquer this disease. To date, however, with the notable exception of hypocholesterolemic agents and risk-factor modifications, precious little has been achieved on this front.

Current pharmacological research in the field of cardiovascular disease prevention is focusing on potential targets at various steps of atherogenesis. The newer agents under development can be divided into three broad categories, namely:

1. anti-inflammatory agents and immunomodulators;
2. inhibitors of cholesterol synthesis and modulators of intermediary metabolism;
3. antiatherosclerosis vaccines.

ANTI-INFLAMMATORY AGENTS AND IMMUNOMODULATORS

Inhibitors of plaque formation

The first step in the development of an atherosclerotic plaque is the formation of the fatty streak—a collection of lipid-rich macrophages or foam cells along with some T cells. This is the reversible stage in atheroma development. Fatty-streak formation is triggered by hypercholesterolaemia and other risk factors that cause focal activation of endothelium in large and medium-sized arteries. Excess low-density lipoprotein cholesterol (LDL-C) infiltrates the artery and is retained in the intima, especially at sites of haemodynamic strain. This LDL-C undergoes oxidative and enzymatic modifications leading to formation of oxidised LDL-C as well as further activation of the endothelium. The activated endothelium expresses various adhesion molecules (eg, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1)). Circulating leucocytes, especially monocytic cells, adhere to this activated endothelium and enter the developing atherosclerotic plaque. The monocytes entering the plaque differentiate into macrophages under the influence of macrophage-colony stimulating factor. The differentiation of a monocyte into a macrophage is associated with upregulation of pattern recognition receptors of innate immunity—the scavenger receptors and toll-like receptors. The scavenger receptors on macrophages take up modified LDL particles, and the macrophage gradually evolves into a lipid-laden foam cell.

The toll-like receptors bind bacterial proteins, stress proteins (eg, HSP-60) and DNA motifs. Oxidised LDL also activates toll-like receptors which, in turn, initiate a signal cascade leading to cell activation.[3]

Each of these steps in atheroma formation is a potential target for antiatherosclerotic treatment (box 1). Strategies that can inhibit and reverse fatty-streak formation include hypocholesterolemic agents (eg, 3-hydroxy-3-methyl glutaryl coenzyme A (HMGCoA) reductase inhibitors) as well as control of other modifiable risk factors such as hypertension and dysglycaemia.

Agents that may inhibit conversion of LDL-C to oxidised LDL-C are also attractive targets. While some herbal preparations, probucol and polyphenols such as resveratrol may be acting through this mechanism, the effectiveness of antioxidant agents per se has so far been disappointing.[4 5]

Inhibition as well as antagonism of cell adhesion molecules such as ICAM-1 and VCAM-1, expressed by the activated endothelium, is another potential target. Probucol may be acting partly by reducing VCAM-1.[6] Poly (ADP-ribose) polymerase (PARP) inhibitors have been shown to reduce ICAM-1 expression.[7] Endothelial expression of ICAM-1 and VCAM-1 is also reduced by the arginase inhibitor L-norvaline.[8] Further, the stimulation of ICAM-1, VCAM-1 and E-selectin by vascular endothelial growth factor (VEGF) has been shown to be suppressed by the phosphatidylinositol 3′-kinase mediated pathway, making regulation at this level a potential target for retarding atherosclerosis.[9] VEGF blockade per se is another avenue under active research.

The development of the monocyte into a macrophage requires a chemokine milieu, including macrophage-colony-stimulating factors. Lack of monocyte chemoattractant protein-1 has been shown to be associated with slower atherosclerosis in mice.[10] In fact, the entire atherosclerotic process—right from the stage of immune cell migration and activation to plaque lysis and rupture—is modulated by a balance between pro- and anti-inflammatory cytokines.[11] Any success in shifting this balance to cause an absolute or relative deficiency of proinflammatory cytokines, without adversely affecting the body's defence mechanism, would be groundbreaking. Work on this front to date has been limited to animal studies.

The macrophage receptors are the next sequential targets. Ligand of type B scavenger receptors, expressed on macrophage, is in the early stages of development (drug EP80317).[12] Genetic removal of a molecule in the toll-like receptor signal pathway has been shown to inhibit atherosclerosis in mice.[13]

Inhibition of T-cell-mediated immunity

The presence of antigen-specific T cells in atheromatous lesions points to the role of cell-mediated immunity in the progression of atherosclerosis.[14] Antigens presented by macrophages and dendritic cells trigger activation of antigen-specific T cells. CD4+ T cells reactive to oxidised LDL, heat shock protein-60 (HSP-60) and Chlamydia have been cloned from human lesions.[11 15] Activated T cells produce Th1 cytokines (eg, interferon γ) which activate macrophages and vascular cells. Regulatory T cells modulate the process by secreting anti-inflammatory cytokines (such as interleukin-10 and transforming growth factor β). T-cell-mediated immunity is under tonic inhibition by TGF-beta and IL-10.

The extent of atherosclerosis is reduced in mice lacking interferon-gamma or its receptor and on inhibition of the Th1 pathway.[16 17] IL-10 and interferon γ are potential targets under investigation. Upregulation of TGF-beta is another potential mechanism for antiatherosclerosis.[18] The fact that antigen-specific immunity has a role in atherogenesis also gives us hope that atherosclerosis may be amenable to immunisation, provided appropriate antigenic targets are identified.

Plaque stabilisers

Apart from inhibiting the process of plaque formation, another novel strategy to reduce atherosclerosis related adverse

Box 1 Antiatherosclerosis: targets for anti-inflammatory agents and immunomodulators

I. Inhibition of plaque formation
   a. Inhibition of low-density lipoprotein cholesterol oxidation
   b. Cell adhesion molecules
      — Inhibition/antagonism
      — Modulation/blockade of vascular endothelial growth factor
   c. Cytokine modulation in favour of anti-inflammatory cytokines
   d. Macrophage receptors
      — Ligands/genetic abrogation
II. Inhibition of T-cell-mediated immunity
   a. Inhibition/receptor blockade of interferon-γ
   b. Upregulation of transforming growth factor β/interleukin-10
   c. Target antigens for immunisation against atherosclerosis
III. Plaque stabilisation
   a. Inhibition of matrix metalloproteinases (eg, matrix metalloproteinases 2 and 9)
   b. Activation of tissue inhibitors of metalloproteinases
   c. Lipoprotein-associated phospholipase A2 inhibition
IV. Miscellaneous agents/targets (agents/strategies with overlapping anti-inflammatory and lipid modulating effects)
   a. Risk factor modification
   b. 3-Hydroxy-3-methyl glutaryl coenzyme A reductase inhibition (with associated pleiotropic effects)
   c. Cannabinoid-1 receptor antagonism/cannabinoid-2 receptor activation
   d. Certain ACE inhibitors/lipophilic β blockers/oral antidiabetics (antiatherosclerotic effects over and above primary risk factor modifying action)
   e. Nutraceuticals (varying extent of antioxidant, anti-inflammatory, lipid modulating and/or antiarrhythmic properties)

events is to stabilise the plaque by increasing its fibrous content and by inhibiting its disruption (box 1). Efforts here are focused on inhibiting specific matrix metalloproteinases (such as MMP-2 and MMP-9) and activating tissue inhibitors of metalloproteinases (TIMP). Probucol has been shown to reduce MMP-2 and 9, while PARP inhibitors increase levels of TIMP-2, thus increasing the collagen content of plaque.[19 20] Darapladib, an orally active lipoprotein-associated phospholipase A2 (Lp-PLA2) inhibitor, currently in phase-III clinical trials, selectively inhibits Lp-PLA2 in plasma as well as plaques, thereby reducing plaque vulnerability.[21]

Another drug in stage III clinical trials is thromboxane A_2 receptor antagonist Terutroban which, apart from its antiplatelet action, also inhibits the action of prostaglandin endoperoxides and isoprostanes on inflammatory cells, thus having an antiatherosclerotic effect.[22]

Yet another novel target for retardation of atherosclerosis is modulation of the endocannabinoid system. The cannabinoid 1 (CB1) receptor antagonist, Rimonabant, has been shown to have a favourable effect on lipid profile as well as reduce markers of inflammation (eg, CRP). However, the STRADIVARIUS Trial demonstrated no difference in the percentage atheroma volume in coronary artery disease (CAD) patients treated with Rimonabant.[23] Also, the safety of Rimonabant, with respect to its psychiatric side-effect profile, is an issue of concern. Activation of CB2 receptor has been shown to have anti-inflammatory and immunosuppressive effects, and retard atherosclerosis in mice.[24]

Substantial attention has also been focused on nutraceuticals—a term derived from 'nutrition' plus 'pharmaceutical'—referring to food-derived substances that have medical or health benefits. It includes a heterogenous group of substances with varying extents of antioxidant, anti-inflammatory, lipid-modulating and/or antiarrhythmic properties. Candidate substances include antioxidant vitamins C and E, vitamin D, folic acid, niacin, ω3 fatty acids, plant-derived flavonoids and polyphenols such as resveratrol.[25 26] Niacin is a classic example of a nutraceutical that we have learnt to concentrate and is among the few effective strategies to raise HDL-C levels.

While short-term beneficial endothelial effects have been reported with antioxidant vitamins C and E, clinical outcome trials have largely been negative—if not outright harmful. It may be that some amount of oxidant stress is important to stimulate vascular protective responses, such as upregulation of endothelial NO synthase. These antioxidants may also be blunting ischaemic preconditioning—a phenomenon responsible for increasing tolerance to major ischaemia.[25 26]

While an improvement in endothelial function has also been attributed to vitamin D in several studies, clinical benefits remain to be demonstrated. Folic acid and vitamin B_6/B_{12} have failed to show any sustained improvement in endothelial function on prolonged administration or a mortality benefit.[25]

The ω3 fatty acids include the plant-derived α-linoleic acid (ALA) and marine-derived eicosapentaenoic acid and docosahexaenoic acid (EPA and DHA). Both, improvements in endothelial function and mortality benefits have been demonstrated, especially with EPA and DHA, although the contribution in mortality reduction may be more from their antiarrhythmic rather than antiatherosclerotic propreties. Further studies to identify individuals likely to derive maximum benefits as well as to define appropriate dosage are required.[25 26]

Studies with plant-derived flavonoids have largely used flavonoid-rich food such as cocoa and black or green tea, rather than isolated flavonoids. While there is some indication of a benefit, it is difficult to ascribe what component is due to the flavonoid content per se. Resveratrol, a phytopolyphenol present in wine (especially red wine), has been shown to improve endothelial function and is possibly cardioprotective, though long-term clinical outcome studies are lacking.[25]

INHIBITORS OF CHOLESTEROL SYNTHESIS AND MODULATORS OF INTERMEDIARY METABOLISM

The most successful antiatherosclerotic strategy so far has been the inhibition of cholesterol synthesis, though the most successful agents in this class (ie, statins) also exert part of their action through a pleiotropic anti-inflammatory effect. The focus of current research is on ways to enhance the reverse cholesterol transport process (box 2). Drugs that raise high-density lipoprotein-cholesterol (HDL-C) are available but have limitations. Fibric acid derivatives such as gemfibrozil and fenofibrate raise HDL only by 10–15%. While large doses of niacin can raise HDL by up to 25% or more, these doses are difficult to tolerate.

A primary target enzyme for increasing HDL-C is the cholesteryl ester transfer protein (CETP), which facilitates the transfer of cholesteryl ester from HDL-C to LDL-C and very-low-density-lipoprotein-C. However, success on this front has so far been elusive. Although a CETP inhibitor, torcetrapib, was developed, it was found to have no significant effect on progression of atherosclerosis, despite a significant increase in HDL levels.

Box 2 Targets for modulators of intermediary metabolism

I. Low-density lipoprotein cholesterol reduction
   a. 3-Hydroxy-3-methyl glutaryl coenzyme A reductase inhibition—for example, statins
   b. Squalene synthase and epoxidase inhibition
II. Reverse cholesterol transport enhancement
   a. Fibric acid derivatives/niacin
   b. Cholesteryl ester transfer protein inhibition—for example, anacetrapib/dalcetrapib
   c. Increasing Apo-A1/liver X receptor agonism/lecithin cholesterol acyltransferase activation/high-density lipoprotein mimetics
III. Acyl-coenzyme A: cholesterol acyltransferase inhibition
   a. Non-selective acyl-coenzyme A: cholesterol acyltransferase-1 and acyl-coenzyme A: cholesterol acyltransferase-2 inhibition
   b. Selective acyl-coenzyme A: cholesterol acyltransferase-2 inhibition
IV. Inhibition of hepatic lipoprotein assembly
   a. Apo-B antisense oligonucleotides
   b. Microsomal triglyceride transfer protein inhibitors
V. Miscellaneous agents with dual anti-inflammatory and lipid modulating properties (detailed in box 1).

Possible reasons for this failure could be the associated increase in blood pressure or dysfunctionality of the increased HDL-C.[27] The results of the recently concluded phase-III DEFINE trial with another CETP inhibitor anacetrapib are encouraging. The drug was associated with a marked reduction in LDL-C (39.8%) and a 138.1% additional increase in HDL-C, when administered to high-risk or known CAD patients already taking statins. Unlike tocetrapib, anacetrapib was not associated with an increase in blood pressure or aldosterone levels. Large clinical outcome trials would now be required to assess the morbidity and mortality benefits of this drug in high-risk individuals.[28]

Phase III clinical trials are also ongoing with another CETP inhibitor, dalcetrapib. Dalcetrapib has a unique mode of action: it induces a conformational change in the enzyme rather than forming a non-productive CETP/HDL-C complex. The drug has so far not been found to be associated with activation of the renin–angiotensin–aldosterone system or an increase in blood pressure.[29]

Apart from CETP inhibition, other experimental modalities aimed at increasing HDL-C levels include methods to increase apolipoprotein (Apo) A1 levels (such as ApoA1 infusions), upregulation of ATP Binding Cassette Transporter A1 and G1 by liver X-receptor agonists, enhancing activity of lecithin cholesterol acyltransferase (LCAT) and development of HDL mimetics.

A discussion on reverse cholesterol transport as a target for antiatherosclerosis would be incomplete without mentioning two concerns that plague this novel concept. First, while observational evidence supporting the inverse relationship between HDL-C levels and coronary artery disease is overwhelming, results from interventional trials are largely equivocal. A recent meta-analysis of 108 clinical trials showed that once adjustments for changes in LDL-C were made, there was no association between treatment-induced change in HDL-C and risk for CAD, CAD events or total deaths.[30]

Also, concerns have been raised that artificially increasing HDL-C may increase the non-cardiovascular mortality. A recent meta-analysis found a positive association between HDL-C increase and non-cardiovascular mortality. However, when the ILLUMINATE trial with torcetrapib was excluded, there was no correlation between the two. Thus, this association is possibly attributable to the off-target effects and drug characteristics of torcetrapib per se, rather than an increase in HDL-C.[31]

Another target enzyme under active investigation is the acyl-coenzyme A: cholesterol acyltransferase (ACAT), which esterifies cholesterol in a variety of tissues (box 2). Two isoforms of this enzyme have been identified: ACAT1 and ACAT2. While ACAT1 is present in many tissues including macrophages, ACAT2 is present in intestinal epithelial cell and hepatocytes. Theoretically, inhibition of ACAT1, by blocking the esterification of cholesterol, could prevent the transformation of macrophages into foam cells and slow the progression of atherosclerosis. Inhibition of ACAT2 would be expected to decrease serum lipid levels. However, the ACTIVATE trial with the non-selective ACAT inhibitor pactimide found it to have paradoxically proatherogenic effects.[32] This could be because by blocking esterification of cholesterol, ACAT1 increases the level of free cholesterol in macrophages which, beyond a stage, causes apoptosis of the macrophage. Cellular necrosis within the atherosclerotic lesion could further aggravate inflammation.[32] Another potent ACAT inhibitor, avasimibe, also failed to retard coronary atherosclerosis as assessed by intravascular ultrasound; it also showed a statistically significant rise in LDL-C.[33]

After the failure of non-selective ACAT inhibitors, efforts are now on to develop ACAT2 selective agents. Beauveriolides are an interesting class of agents under development. These fungal metabolites have been shown to inhibit atherosclerosis in mice. Importantly, different analogues show different selectivity towards ACAT1 and ACAT2, making them potential agents for selective ACAT2 inhibition.[34]

Another novel approach in intermediary metabolism includes reduction in very-low-density-lipoprotein-C and LDL-C by inhibiting hepatic lipoprotein assembly using apolipoprotein-B (Apo-B) antisense oligonucleotides and inhibitors of microsomal triglyceride transfer protein.[35] Unlike microsomal triglyceride transfer protein inhibitors, Apo-B antisense oligonucleotides do not appear to increase the risk of hepatic steatosis.

Enzymes downstream to HMG-CoA reductase (eg, squalene synthase and epoxidase) are also potential targets for enhanced LDL-lowering especially for statin intolerant patients or as adjuvants to statins.[36] A phase-III clinical trial with squalene synthase inhibitor Lapaquistat had to be aborted owing to concerns regarding its hepatotoxicity.

Apart from these, several agents such as statins, peroxisome proliferator activated receptor γ (PPARγ) agonists, CB1 receptor antagonists (rimonabant), etc have dual anti-inflammatory as well as lipid-modulating properties.

ANTIATHEROSCLEROSIS VACCINE

The concept of immunisation against non-communicable diseases is an intriguing one. Target diseases against which vaccine development is being attempted include multiple sclerosis, Alzheimer's disease, type 1 diabetes mellitus, rheumatoid arthritis and certain cancers. The idea for antiatherosclerosis vaccine takes root in the observation that antigen-specific T cell immunity has a role in atherogenesis.

Experimental data suggest that animals lacking functional T cells or different pro-inflammatory cytokines exhibit various degrees of resistance to atherosclerosis.[16 17] Efforts are ongoing to identify specific plaque antigens recognised by T cells which may act as vaccine targets.

Oxidised LDL-specific T cells have been demonstrated in circulation as well as within atherosclerotic plaques. When hypercholesterolaemic rabbits were immunised with oxidised LDL, the immune response to oxidised LDL was expected to cause a rapid progression in atherosclerosis. Paradoxically, this vaccine produced significant antiatherosclerotic effects, possibly by inducing tolerance to the antigen.[14] The retardation of atherosclerosis by inducing tolerance to oxidised LDL has subsequently been demonstrated in other animal models as well.[37 38]

Oxidised LDL has been at the forefront of antiatherosclerosis vaccine development. Efforts are on to derive specific antigens from this complex particle. Possible antigens include oxidised phospholipids and aldehyde-modified Apo-B 100 peptides.[14] Heat-shock proteins, expressed in response to severe stress, are another potential target for vaccine generation,[39] as is the enzyme cholesterylester transfer protein (box 3).[40] However, the

Box 3 Potential targets for antiatherosclerosis vaccine

I. Oxidised low-density lipoprotein and subparticles thereof
   — Oxidised phospholipids
   — Aldehyde modified Apo-B100 peptides
II. Heat-shock proteins
III. Cholesteryl ester transfer protein
IV. *Chlamydia pneumoniae*

development of an antiatherosclerosis vaccine is still in conceptual stages; even after appropriate antigen selection, a number of hurdles remain, including mode of delivery and side effects such as associated immunosuppression. The duration for which the induced immunity, if any, will last is also a crucial factor determining the success of any future vaccine. Atherosclerosis being a slow but relentless process, any induced immunity will have to last for a considerable duration in order to translate into morbidity and mortality benefits.

With cardiovascular disease being the single leading cause of death worldwide, the development of a safe and successful antiatherosclerosis strategy (possibly employing a combination of agents acting at various levels) will indeed be a major 21st-century achievement.

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