Review

Atherosclerosis: from biology to pharmacological treatment

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Abstract

A recent explosion in the amount of cardiovascular risk has swept across the globe. Primary prevention is the preferred method to lower cardiovascular risk. Lowering the prevalence of obesity is the most urgent matter, and is pleiotropic since it affects blood pressure, lipid profiles, glucose metabolism, inflammation, and atherothrombotic disease progression. Given the current obstacles, success of primary prevention remains uncertain. At the same time, the consequences of delay and inaction will inevitably be disastrous, and the sense of urgency mounts. Pathological and epidemiological data confirm that atherosclerosis begins in early childhood, and advances seamlessly and inexorably throughout life. Risk factors in childhood are similar to those in adults, and track between stages of life. When indicated, aggressive treatment should begin at the earliest indication, and be continued for many years. For those patients at intermediate risk according to global risk scores, C-reactive protein, coronary artery calcium, and carotid intima-media thickness are available for further stratification. Using statins for primary prevention is recommended by guidelines, is prevalent, but remains under prescribed. Statin drugs are unrivaled, evidence-based, major weapons to lower cardiovascular risk. Even when low density lipoprotein cholesterol targets are attained, over half of patients continue to have disease progression and clinical events. Though clinical evidence is incomplete, altering or raising the blood high density lipoprotein cholesterol level continues to be pursued. The aim of this review is to point out the attention of key aspects of vulnerable plaques regarding their pathogenesis and treatment.

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1 Introduction

Atherosclerosis is a chronic inflammatory reaction of the vascular wall in response to dyslipidemia and endothelial distress involving the inflammatory recruitment of leukocytes and the activation of resident vascular cells.[1] The chronic inflammation of arterial vascular wall leading to multifocal plaque development.[2,3] Most plaques remain asymptomatic (subclinical disease), some become obstructive (stable angina) but a few become thrombosis-prone (vulnerable) and lead to atherothrombotic events, such as acute myocardial infarction (AMI),[3] stroke[4] and lower limb ischemia.[5]

Atherothrombotic cardiovascular disease (CVD) is a leading cause of death and disability not only in rich countries but globally and, as such, has a large economic and public health impact.[6] The mortality of atherothrombotic CVD has fallen dramatically in the past decades, and the prolonged survival with chronic disease explains why the prevalence, burden, and costs of this disease remain high.[7,8]

Due to the burden of CVD, identification of patients at risk of cardiovascular events is needed. Serum biomarkers regarding the vulnerable plaque contain important predictive information for future cardiovascular events, also in territories of the vascular tree.[5] Novel circulating biomarkers may provide incremental prognostic information,[9,10] but their clinical utility remains to be established.[11,12] Despite great promise, genetic testing for “susceptibility” has not yet proven useful for risk stratification in clinical practice.[13]

An alternative strategy of potentially greater impact would be assessment of subclinical (asymptomatic) atherosclerosis as proposed in recent guidelines and, in particular, detection of disease activity and plaques of the high-risk “vulnerable” type.[5,14–16] Such lesions are most often hidden in the arterial wall and not diagnosed until it is too late.[17]

The concept of plaque stabilization was introduced several years ago to explain how acute coronary events could be reduced by lipid-lowering therapy without concomitant regression in coronary atherosclerosis assessed by angiography.[18]
It is now well-established that the risk of thrombosis depends more on plaque composition than on the degree of luminal obstruction seen by angiography. However, whether it will be possible to detect prospectively plaques at risk of thrombosis, target therapy to those lesions and eventually reduce the risk of clinical events, remain to be proven.\[19\]

In recent years, studies using advanced imaging technologies have provided insights into atherosclerotic plaque development and its progression to vulnerable plaque and rupture as a cause for acute atherothrombosis leading to AMI or stroke.\[20–23\]

Inflammation activity can be evaluated at the sub (cellular) level using in vivo molecular magnetic resonance imaging (MRI). Recent progress in contrast-enhanced molecular MRI to visualize atherosclerotic plaque inflammation with various contrast agents has been developed. Transfer of these molecular MRI information to the clinic is important for to develop new diagnostic tools and potential therapies.\[24\]

2 Inflammation

Atherosclerosis is an inflammatory disease in which inflammatory activity is not confined to just a few atherosclerotic lesions but is present, more or less, in all such lesions throughout the vascular tree. In contrast, vulnerable plaques are relatively rare, and inflammation may play a causal role in plaque rupture only if located within a thin fibrous cap, i.e. the microstructure of the plaque needs to be permissive for the rupture. Thus, although plaque inflammation may be useful as a marker of disease activity, it is probably not useful as a stand-alone marker for plaque vulnerability.

2.1 Inflammatory cells in atherosclerotic lesions

The fibrous caps of vulnerable plaques contain abundant blood derived leukocytes, including monocytes, macrophages and T-lymphocytes. Of the T-cells, CD4+ T-helper (Th) lymphocytes are the most prominent.\[25\] Initially present in a Th0 ground state, after engagement of the T-cell receptor, naïve T-cells can differentiate to Th1 cells, which secrete and respond to interferon-γ (IFN-γ) or to Th2 cells, which secrete and respond to interleukin-4 (IL-4), interleukin 10 (IL-10) and interleukin 13 (IL-13). Interestingly, IFN-γ blocks Th2 differentiation while IL-4 blocks Th1 differentiation so there is a tendency for the T helper response to become polarized.\[26\] On the contrary, several populations of regulatory T-cells (Tregs) and their characteristic cytokines, including IL-10 and transforming growth factor (TGF)-β, consistently reduce atherosclerosis and favors stable plaque morphology. Thus, immunomodulation which aims to change the T-helper cell milieu and promote Tregs, seems an attractive possibility. T-cells promote plaque vulnerability locally through their effects on macrophages and foam-cell macrophages, which are ultimately mostly derived from blood monocytes. Classical activation by pro-inflammatory cytokines, including IFN-γ, amplifies production of pro-inflammatory mediators, MHC-II related antigens and extracellular proteases, thereby tending to promote inflammation and tissue destruction.\[27,28\] By contrast, alternative activation of macrophages with IL-4 or IL-13 suppresses production of pro-inflammatory cytokines, MHC-II related antigens and proteases, and increases secretion of connective tissue growth factors, thereby promoting granuloma formation and tissue repair.

2.2 Inflammatory and anti-inflammatory cytokines

It is now increasingly appreciated that the immune response—besides being “atheroprotective” can be counter-balanced by “atheroprotective” cytokines. For instance, inhibition of TGF-β signaling using blocking antibodies accelerated atherosclerosis and induced a vulnerable plaque phenotype in mice.\[29\] In human carotid atherosclerotic plaques, elevated expression of the TGF-β signaling pathway has been associated with higher collagen and smooth muscle cells (SMC) content and a more stable plaque phenotype.\[30\]

2.3 Chemokines and growth factors

Chemokines are a large family of small related cytokines that regulate cell trafficking of leukocytes to areas of injury. To date, 42 chemokines and 18 chemokine receptors have been identified. Chemokines have been grouped into four subfamilies, CXC, CC, CX3C and C chemokines. A set of independent studies has convincingly revealed that CCR5, the receptor for the platelet-derived chemokine RANTES/CCL5, drives a pro-inflammatory Th1-type immune response and supports advanced atherosclerotic plaque formation, whereas its deficiency is atheroprotective and confers features of plaque stability, an effect probably residing in bone marrow-derived cells. Lesions with CCR5 deficiency showed a marked reduction in matrix metalloproteinase (MMP)-9 expression and increase in collagen accumulation.\[31,32\]

Expression of IL-8 and IL-6 was also correlated with a vulnerable plaque phenotype in human carotid atherectomy specimen.\[33\] This effect is probably mediated through a local activation of perivascular mast cells, as they increase vascular leakage, induce plaque hemorrhage, macrophage apoptosis and leukocyte infiltration through the IL-8 receptor CXCR2.\[34\] Notably, the pleiotropic chemokine-like cytokine and CXCR2 agonist macrophage migration inhibitory factor (MIF) is up-regulated during atheroprogression, is abundantly present in advanced complicated lesions and can induce MMP-9 expression.\[35\] In this context, it is noteworthy that MIF expression in human carotid atherectomy specimens has been identified as one of the most powerful predictors of systemic cardiovascular events.\[36\] Another important mediator,
monocyte chemotactic protein-1 (MCP-1/CCL2), is also expressed in atherosclerotic lesions, where it participates in monocyte recruitment. Beyond its importance in angiogenesis, vascular endothelial growth factor (VEGF) is expressed along with its receptors in atherosclerotic plaques, and may contribute to plaque progression and expansion of the vulnerable phenotype after systemic application.

3 Platelets

Platelet adhesion under conditions of high shear stress, as occurs in stenotic atherosclerotic arteries, is central to the development of arterial thrombosis; therefore, precise control of platelet adhesion must occur to maintain blood fluidity and to prevent thrombotic or hemorrhagic complications. Beyond their great role in hemostasis and thrombosis, platelets contribute to the formation, progression and exacerbation of atherosclerotic plaques through their secretory functions and as important modulators of inflammatory and immune responses, but the role of platelets and coagulation in plaque stability remains to be elucidated. Most notably, plaque progression and inflammation is promoted by deposition and synergistic functions of platelet chemokines, e.g., RANTES/CCL5, on the arterial surface triggering monocyte arrest and macrophage infiltration. Clinically, elevated plasma levels of CCL5 specifically predict refractory symptoms and future events in unstable angina pectoris. Thus, platelets and coagulation appear to have hitherto underappreciated effects on plaque stability and subsequent complications. However, experimental studies strongly support the validity of anti-inflammatory approaches to promote plaque stability.

4 Endothelial dysfunction

During the last two decades, accumulating evidences has described the vascular endothelium as an active endocrine, paracrine, and autocrine organ, indispensable for the maintenance of vascular homeostasis. Altered homeostasis induced by various stimuli may cause localized alterations, or “endothelial dysfunction”, of the antithrombotic properties, vascular tone, heightened leukocyte adhesion, and increased production of cytokines and growth factor. The dysfunctional state of the endothelium links to increased permeability of particles such as low density lipoprotein cholesterol (LDL-C) and inflammation of the vascular wall. Based on these two processes alone, it is conceivable that dysfunctional endothelium contributes to the development of a lipid-rich and inflamed plaque. Much of these changes have been attributed to the influence of biological and physical risk factors, such as hypercholesterolemia and shear stress, respectively, leading to an alteration in the endothelial gene expression profile. This translates into an activation of oxidative stress pathways such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, the endogenous endothelin system, and most importantly, a down regulation of nitric oxide (NO) bioavailability. NO arises from the conversion of L-arginine to L-citruline in the presence of NADPH, a reaction catalyzed by NO synthase (NOS), which exist as three isoforms. Superoxide anions react with NO to form peroxynitrite, which then interacts with tetrahydropterin (BH4), decreasing its availability as a co-factor for eNOS. Under these circumstances, eNOS becomes no longer the source of NO but rather superoxide anions (so-called “eNOS uncoupling”). The stimulation of the oxidative stress pathway therefore leads to a self-perpetuating cycle of NO depletion. The cardiovascular consequences of this process have been well-established in experimental models of pharmacological inhibition of NO synthase, which demonstrated aggravation of atherosclerotic plaque formation. Conversely, intervention aimed at up-regulating the NO pathway demonstrated an attenuation of the atherosclerosis process in a predisposing environment.

Population-based studies identified coronary endothelial dysfunction as an independent predictor of major adverse cardiac events, and noted that preserved endothelial function attenuates the risk of future events in patients with high plaque burden. The causal aspect of this association is strengthened by the fact that acute triggers of cardiovascular events are remarkably linked to endothelial dysfunction. For instance, cardiovascular events follow a circadian periodicity with a peak incidence in the early morning hours. Intriguingly, patients with CVD who suffered from acute coronary syndrome (ACS) show a loss of diurnal variation in endothelium-dependent vasodilation that may counteract potentially adverse diurnal variations in other biological factors.

In the presence of an activated and dysfunctional endothelium, platelet activation and aggregation is enhanced due to reduced production of NO, prostacyclin (PGI2) and likely ecto-adenosine phosphohydrolase (ADPase)/nucleoside triphosphate diphosphohydrolase (NTPDase-1)/CD39. The release of peptides such as serotonin and thrombin from activated platelets leads to further potent vasoconstriction and enhances the outlined dynamics. Acute thrombotic occlusion is furthermore favored by reduced production of tissue plasminogen activator and thrombomodulin and an increased production of tissue factor by a dysfunctional endothelium.

Particularly in the setting of severe vasoconstriction, shear forces at the plaque can increase to such a level that it would cause marked endothelial damage followed by platelet deposition and thrombus formation. Detachment of endothelial cells from the collagen IV-rich basement membrane in these
areas is favored by the production of type IV collagenases (MMP-2 and MMP-9) by activated endothelial cells and inflammatory cells.\textsuperscript{58,59} Finally, low and oscillatory shear stress in the downstream part of the atherosclerotic plaque can induce endothelial cell apoptosis and detachment with mural thrombus formation by modulation of \(\alpha/\beta\)-integrin expression and caspase-3 activity.\textsuperscript{60,61}

5 Angiogenesis and plaques instability

The progression of atherosclerotic plaques is associated with the appearance and growth of vasa vasorum. In humans plaque microvessel content increases with plaque progression and is likely stimulated by plaque hypoxia, hypoxia-inducible factor (HIF) signaling, reactive oxygen species (ROS) or other inflammatory signals.\textsuperscript{62} The presence of plaque hypoxia is primarily determined by plaque inflammation (increasing oxygen demand), while the contribution of plaque thickness (reducing oxygen supply) seems to be a minor factor. Plaque microvessels are immature and fragile and the distorted integrity of microvessel endothelium likely leads to intra-plaque hemorrhage and plaques at increased risk for rupture.\textsuperscript{63}

The histological detection of intra-plaque hemorrhage is associated with plaque rupture.\textsuperscript{64} However, it remains to be demonstrated, whether the intra-plaque haemorrhage from neo-vessels triggers plaque rupture or vice versa. Nevertheless, adventitial microvessels are clearly related to atherosclerotic disease and currently provide the only acceptable option to study anti-angiogenic interventions in animal models of atherosclerosis. The inhibition of angiogenesis using two independent angiogenesis inhibitors endostatin and TNP-470 resulted in the attenuation of plaque growth in apoE\textsuperscript{-/-} mice.\textsuperscript{65} It has been suggested to use anti-angiogenic therapy for plaque stabilization in humans.\textsuperscript{66}

6 Biomarkers of cardiovascular risk

A good biomarker needs to be specific for disease development or progression, to have a high predictive value for events and, if possible, should reflect successful treatment. Atherosclerosis is a multi-factorial disease and therefore one single biomarker will not likely be sufficient to reach all these objectives. However, with the currently available circulating biomarkers, even the use of multiple biomarkers only adds moderate predictive value to the traditional CVD risk factors.

6.1 High sensibility C-reactive protein

A large body of literature supports the idea that inflammation [and in particular C-reactive protein (CRP)] plays a pivotal role in all phases of atherosclerosis, from the fatty streak lesion formation to the acute coronary event due to vulnerable plaque rupture. Indeed, vascular inflammation contributes to the pathogenesis of atherosclerosis, and later in the disease process, it is a major determinant for the ACS. There are various inflammatory markers that have been shown to predict cardiovascular events. These include high-sensitivity CRP (hs-CRP), a simple downstream marker of inflammation, recently emerged as a major cardiovascular risk factor.\textsuperscript{67} Elevated baseline concentrations of hs-CRP are associated with the risk of atherosclerotic events in general populations and show a predictive value even in terms of secondary prevention, both in patients with chronic stable angina and ACS.\textsuperscript{68,69} If CRP is just a biomarker or also causally involved in plaque formation is still under debate.\textsuperscript{70,71}

6.2 Chemokines and cytokines

Chemokines and cytokines seem very promising targets,\textsuperscript{72} and there are also other interesting reports in this regard on e.g., tissue metalloproteinases, homeostatic factors and myeloperoxidase,\textsuperscript{73–75} but they all still need validation and confirmation. Given these results, there is a pressing need for more specific and prognostic biomarkers to be added to the established risk factors to optimize risk prediction. Currently, studies are ongoing that use alternative sources for biomarker discovery for the progression of atherosclerotic disease. The local atherosclerotic plaque is considered as a source for biomarkers to predict future systemic adverse events.\textsuperscript{76}

7 Plaque stabilization: clinical trials, current treatments and future perspectives

Several therapeutic strategies have been tested for the pharmacological stabilization of vulnerable plaques and some have entered current treatment guidelines.\textsuperscript{77} For example, the familiar atherosclerotic treatment study (FATS) demonstrated only a very moderate reduction in angiographic stenoses in patients receiving lovastatin plus colestipol and in those randomized to niacin plus colestipol compared with those receiving standard care.\textsuperscript{78} In contrast to these angiographic results, patients assigned to the lipid-lowering strategy experienced an overall reduction of 73% in CVD events (death, AMI or ischemic events requiring intervention) compared with usual care. Similar data were found in the St. Thomas atherosclerosis regression (STAR) study randomizing patients to cholestyramine.\textsuperscript{79} Based on these data, the hypothesis was raised, that lipid-lowering therapy may stabilize arteriosclerotic lesions and decrease the likelihood of plaque rupture thus, e.g., reducing the incidence of AMI. With respect to therapeutic strategies to stabilize such lesions, however, current knowledge is still limited.
Statins represent the primary therapy used to reduce atherosclerosis and cardiovascular events such as myocardial infarction (MI), stroke, transient ischemic attacks (TIA), and in patients undergoing carotid endarterectomy. Statins have been shown to stabilize atherosclerotic plaques by reducing plaque lipids and thrombogenicity, improving endothelial function and by their anti-inflammatory action. To date, two studies have examined the effect of statin therapy on plaque inflammation and plaque stability. In the first study, patients received pravastatin or no treatment three months before surgery. Plaques were then removed and analyzed for lipid content, inflammatory cells and collagen content. This study demonstrated that patients receiving pravastatin exhibited significantly higher collagen content and less inflammatory cells, suggesting that these plaques were more stable than plaques from untreated patients. The atorvastatin and thrombogenicity of the carotid atherosclerotic plaque (ATROCAP) study randomised patients eligible for two-step bilateral carotid endarterectomy to atorvastatin or placebo for 4–6 months following the first procedure. In this study, post-treatment plaques from patients treated with atorvastatin showed a trend towards fewer macrophages and inflammatory cells than pre-treated plaques, whereas no change was observed in patients receiving placebo. In addition, patients receiving atorvastatin exhibited less tissue factor content in post-treatment plaques, suggesting additional anti-thrombotic effects of atorvastatin. However, many of these changes did not reach significance, most likely due to the small sample size and a high intra- and inter-individual variability. Still, the results of these two studies were consistent with experimental findings that statins, in addition to their lipid-lowering properties, exhibit pleiotropic, anti-inflammatory effects which are likely to contribute to plaque stabilization in treated patients.

Such mechanisms could play a role in the large reduction of CVD events in clinical trials. Overall, immune- and histochemical studies on endarterectomy specimen have contributed to our understanding of plaque stabilization but due to the small number of patients and limitations of the technique, this approach is hard to prove a plaque stabilization effect of a single drug.

Other studies have used intravascular ultrasonography (IVUS) to invasively characterize plaques of treated patients. The German Atorvastatin Intravascular ultrasound (GAIN) study demonstrated that hyperechogenicity of plaques significantly increased in statin-treated patients after 12 months compared to usual care. However, decreasing plaque growth and hypoechogenicity under atorvastatin did not reach significance, likely due to small sample size. Using virtual histo-logy-intravascular ultrasound (VH-IVUS) technique some trials have shown that statin therapy can increase the fibrous tissue volume, decrease the lipid core and increase the fibro-fatty plaque volume, again suggesting that statin therapy may stabilize atherosclerotic lesions.

Large trials evaluating the effects of aggressive lipid lowering with statins have shown beneficial effects on plaque size and CVD events. In the REVERSAL study, aggressive lipid lowering with atorvastatin 80 mg daily decreased hs-CRP levels and regressed the atheroma as shown with IVUS. Similar findings have been reported for different statins and were generated with other techniques such as magnetic resonance imaging (MRI) and angiography. Moreover, aggressive lipid lowering shows even a clinical benefit in primary prevention in the presence of normal cholesterol levels: the JUPITER study randomized 17,802 healthy subjects with LDL-C less than 3.4 mmol/L and hs-CRP above 2 mg/L to 20 mg rosuvastatin or placebo. Rosuvastatin significantly reduced the incidence of major CVD events in this low-risk group.

ACS is the most urgent clinical indication for plaque stabilization. The PROVE-IT study randomised 4,162 patients with ACS to pravastatin (40 mg daily) or atorvastatin (80 mg daily) and followed them up for 24 months. The incidence for the primary endpoint, consisting of death, AMI, unstable angina and revascularization, was decreased by 16% in the aggressive treatment arm.

The patients deriving most benefit were those who decreased both LDL-C and hs-CRP levels. The ARMYDA-ACS study randomized 171 patients with non-ST elevation ACS to atorvastatin or placebo before percutaneous coronary intervention. One month follow-up revealed a significant decrease in major CVD events in the statin group. These studies contribute to our understanding about the beneficial effects of statins on cardiovascular morbidity and mortality.

**8 Strategies options to enhance cardiovascular risk reduction**

The concept of risk factors, introduced by the original Framingham investigators in 1961, essentially established preventive cardiology. Risk factors are now accepted antecedents of atherosclerosis whose levels predict subsequent cardiovascular events and are targets for therapy. The current approach to cardiovascular risk screening is summarized in a state-of-the-art paper by Berger and associates.

Within the past few years there has been a re-evaluation of reducing risk in the general population, and the central unanswered question is: how can people who will eventually have cardiovascular events be identified and their risk lowered? There is no ideal or “gold-standard” risk equation for assessment, nor a drug-response equation for treatment.
Current issues in primary prevention of coronary heart disease include the long incubation period; methods of evaluating risk in the population; population-based vs. individual risk-based approaches; role and refinement of global risk factor scores; choice and merits of non-traditional risk factors; multiple biomarker panels; imaging techniques in evaluation and ongoing therapy; value of advanced lipid testing; weights given to traditional risk factors, cut-off values and treatment targets, particularly LDL-C goals in guidelines; use of statins in primary prevention; reasons for low patient adherence with evidence-based therapies; causes of “clinical inertia” and lack of physician compliance with guidelines; and the etiologies, extent, and minimization of residual risk. Within this period, there have been several suggestions based upon models, proposals, and clinical protocols contributing to the dialog, enumerated below with additional commentary.

9 Anti-platelet therapy

Anti-platelet therapy may stabilize the vulnerable patient by reducing the amount of local thrombus formation as well as reducing vascular inflammation. Aspirin has been shown to be effective for secondary prevention in patients with established atherosclerotic vascular disease. Out of the four commonly recommended therapies for secondary prevention (statin, aspirin, β-blocker, angiotensin-converting enzyme inhibitor), the combination of statin and aspirin are associated with the greatest reduction in mortality in a case-control analysis. In addition to aspirin, there is also positive evidence for other antiplatelet agents such as clopidogrel, prasugrel and ticagrelor. The Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) trial showed a major reduction in CVD events when clopidogrel was added to aspirin in patients with ACS without ST-segment elevation. The positive effects of prasugrel versus clopidogrel (TRITON trial), and ticagrelor (PLATO study) on CVD events were even greater.

10 Anti-hypertensive therapy

B-blockers have been shown to reduce recurrent AMI, sudden cardiac death and total mortality in patients with AMI in several clinical trials. They reduce heart rate and blood velocity resulting in less turbulent flow and lower wall stress. A recent pooled analysis of four IVUS trials has shown that β-blockers slow the progression of atherosclerosis.

Angiotensin II is a pro-inflammatory cytokine and augments the production of ROS. Blocking angiotensin II has been shown to reduce signs of inflammation in atherosclerotic animals. Renin-angiotensin system (RAS) inhibition also improves endothelial function. Clinical trials like HOPE and ONTARGET studies have shown a reduction in coronary events out of proportion to their reduction in blood pressure supporting the concept of plaque stabilizing effects.

11 Other anti-atherosclerotic therapies

The therapeutic option of lowering LDL-C and VLDL-C while raising high density lipoprotein cholesterol (HDL-C) using nicotinic acid has recently received new emphasis, given the strong inverse relationship between CVD risk and HDL-C. Niacin reduces CVD and the progression of atherosclerosis. The identification of a G-protein coupled receptor for nicotinic acid may yield insights into how this compound leads to a favorable alteration in HDL-C, and to pleiotropic anti-inflammatory effects, and may provide a platform for developing candidate molecules without side effects. Recently, two clinical trials indeed revealed that in statin-treated patients with low HDL-C, high-dose modified-release nicotinic acid, compared with placebo, significantly reduces carotid atherosclerosis within 12 months, and that the use of extended-release niacin caused a significant regression of carotid intima-media thickness when combined with a statin, demonstrating that niacin is superior to ezetimibe.

Artificial HDL-like apoA1 complexes, especially apoA1-Milano have been shown to reduce atherosclerotic plaques. Recently, the large Gruppo Italiano Studio sulla Sopravvenenza dall’Infarto–Prevenzione (GISSI-P) trial has shown omega-3 supplementation to have an additional effect on reducing CVD events. Likewise, peroxisome proliferator-activated receptor (PPAR) agonists have shown plaque stabilizing effects in several studies although the clinical evidence is conflicting. The effects of anti-diabetic thiazolidindiones on plaque stability have been studied. In one study, non-diabetic patients were randomized to placebo or rosiglitazone for six weeks prior to carotid endarterectomy, and after surgery plaques were analyzed. Rosiglitazone significantly reduced CD4-positive lymphocyte content without having an effect on the number of plaque macrophages. However, macrophage activation, as assessed by HLA-DR staining, was significantly reduced which resulted in an increase in collagen type-I content, suggesting that rosiglitazone treatment may convert unstable plaques to more stable plaques. Interestingly, the observed results were independent of the glucose-lowering and lipid-modifying properties of this drug. However, large clinical trials, for example the Rosiglitazone Evaluated for Cardiac Outcomes in Diabetes (RECORD) trial failed to show a reduction in CVD events in rosiglitazone-treated patients with type 2 diabetes, questioning the overall benefit of such plaque stabilizing effects of rosiglitazone.

A new approach to treat plaque inflammation is by...
targeting the activity of lipoprotein-associated phospholipase A2 (Lp-PLA2), which primarily acts on oxidized LDL (Ox-LDL).\textsuperscript{[105]} Ox-LDL in vulnerable plaques is known to generate pro-atherogenic compounds, such as lysophosphatidylcholine and is associated with increased risk of CVD events. Indeed, selective inhibition of Lp-PLA2 with darapladib reduced the development of advanced coronary atherosclerosis in diabetic and hypercholesterolemic swine, decreasing lysophosphatidylcholine content, necrotic core area and frequency of lesions with an unstable phenotype.\textsuperscript{[106]} Darapladib exerted anti-inflammatory effects, reducing the expression of macrophage-and T-cell function-associated genes, such as CCL5 and Cathepsin S. In patients receiving high standard-of-care treatment, the effect of darapladib on coronary atherosclerosis was compared with placebo. While necrotic core size as a secondary endpoint and key determinant of plaque vulnerability continued to expand in patients receiving placebo, this was prevented by Lp-PLA2 inhibition with darapladib.\textsuperscript{[107]} As a co-primary endpoint, changes in number and area of regions with high strain did not differ in the overall study but showed a significant reduction in darapladib treated patients. In the absence of safety concerns, darapladib may be a valuable option for future studies to determine favorable effects on CVD.

12 Ongoing therapeutic strategies

Recent studies have identified strong expression of various matrix metalloproteinases (MMPs) in atherosclerotic lesions and their contribution to fibrous cap thinning by degrading extracellular matrix. This process is controlled by transcription, enzyme processing, enzyme activation, and specific inhibition by tissue inhibitors of metalloproteinases (TIMPs). For instance, lesional over-expression of MMP-9 induces acute plaque disruption and promotes intra-plaque haemorrhage in advanced lesions of ApoE\textsuperscript{-/-} mice.\textsuperscript{[108]} Thus, the development of drugs specifically targeting lesional MMPs, namely MMP-9, may be a valuable therapeutic modality for preventing plaque progression and stabilizing rupture-prone plaques.

The potent elastase Cathepsin S, which co-localizes with sites of elastin degradation in human coronary plaques, has also been implicated in primary atherosclerosis, as evident by reduced plaque size, intimal macrophage and lipid content in cathepsin S-deficient LDLR\textsuperscript{-/-} low density lipoprotein receptor) mice.\textsuperscript{[109]} Moreover, plaques in brachiocephalic arteries of fat-fed cathepsin S-deficient ApoE\textsuperscript{-/-} mice showed fewer ruptures and a more stable plaque phenotype. Using a biotinylated form of its endogenous inhibitor cystatin, active cathepsin S was detected in plaques, especially in macrophages of the shoulder regions. Beyond revealing a role of Cathepsin S in plaque formation, destabilization and rupture, these studies open an interesting option for therapeutic targeting with cystatins.

A highly selective approach to suppress atherogenic and destabilizing functions of the CCR5 ligand CCL5 without clinically relevant side effects has been recently introduced by the disruption of synergistic heteromer formation of the platelet-derived chemokines CCL5 and its interaction partner CXCL4 using a cyclic peptide approach.\textsuperscript{[110]} The treatment with the peptide resulted in a marked inhibition of athero-progression and inflammatory cell content and also appears to exert beneficial effects in myocardial ischemia-reperfusion. This compound has undergone extensive toxicological testing and is currently under clinical testing for CVD. The chemokine-like cytokine migration inhibitor factor (MIF) has been implicated in athero progression and the formation of unstable plaques with MMP-9 expression in mouse and man.\textsuperscript{[35]} Hence, an inhibition of MIF with biologicals, e.g., peptides, or with small-molecule antagonists targeting its CXCR2 chemokine receptor agonism, as currently developed, may be similarly suited to mediate regression of atherosclerosis and stabilization of advanced plaques, as seen with a monoclonal antibody in mouse models.\textsuperscript{[36]} Obviously, the validation of its effectiveness, as for other drugs and candidates, will require vigorous testing and appropriate clinical study settings, e.g., coronary atheroma analysis by IVUS.

It is well accepted that adaptive immunity regulates the extent of pro-atherogenic inflammation and that T-cells can affect the stability of atherosclerotic lesions. Immunization of hyperlipidemic animals with LDL-C preparations or fragments of apoB-100 reduces atherosclerosis, suggesting that vaccination may represent a useful strategy for disease prevention or modulation.\textsuperscript{[111,112]} Studies applying immunization strategies with subcutaneous injections of ox-LDL or native LDL-C yielded a reduction in atherosclerosis even independently of antibody titers to oxidative neoepitopes. It remains to be seen whether oxLDL is the most appropriate antigen for vaccination. Alternative options include aldehyde- modified apoB-100 peptide antigens, oxidized phospholipid antigens, heat shock proteins or other antigens, such as VEGF-receptor 2. It has been shown that immune responses elicited by transferred antigen-loaded dendritic cells directed towards antigens presented by arterial SMCs aggravate atherosclerosis.\textsuperscript{[113]} Presenting antigens, such as LDL-C, ox-LDL or derived peptides in the vascular wall, dendritic cells may instigate or sustain inflammatory T-cell responses driving atherosclerosis. Notably, dendritic cell-based vaccination strategies have been successful in other contexts, such as protecting mice from autoimmunity.\textsuperscript{[114]} This strategy may also apply to the treatment of atherosclerosis and CVD.
In summary, for plaque stabilization there is strong clinical evidence for statins, and also positive results for aspirin and other antiplatelet agents, β-blockers and renin-angiotensin-aldosterone system inhibitors. Also, there is some clinical evidence for PPAR agonists, niacin, omega-3 fatty acids and some HDL raising therapies for plaque stabilization. It can be anticipated that compounds such as cholesteryl ester transfer protein (CETP) inhibitors will be further refined, and others, such as PLA2 inhibitors will be tested in larger scale trials. New targets and strategies, such as vaccination emerge to be validated, while established drugs such as niacin, owing to new formulations and consolidated insights, will receive new attention.

13 Conclusions

Alternatives for improving cardiovascular prevention based upon evolving concepts, new data, and revised goals have changed remarkably in recent years. In primary prevention, traditional risk factors used in combination to generate global scores do not predict risk well enough, nor do they discriminate sufficiently between those who will have cardiovascular events and those who will not. Choosing the best mix of approaches for cardiovascular prevention cannot presently be based upon hard end point data, but partially upon an evidence-based synthesis using inductive reasoning.

Even though the original belief that prevention was cost-ineffective has now been disproven, at least for cardiovascular applications, there has been disappointing progress in effecting successful population-based primary prevention. For truly effective improvements in cardiovascular risk, primary prevention appears necessary as an adjunct to the high-risk strategy traditionally offered to individual patients. Wilkins and Lloyd-Jones explicitly declare that the present paradigm of identifying high-risk individuals alone will never succeed in lowering the risk burden, even without considering further progression of obesity and diabetes.

The magnitude of the problem, pervasive poor cardiovascular health and its importance, has not been fully appreciated. Psychosocial aspects of behavior in embracing and adhering to primary and secondary prevention are receiving greater attention. According to one health belief model, negative health behavior is in part due to the widespread failure of people to accept disease preventive when disease is asymptomatic. Part of the complex belief system involved leads to unrealistic optimism of vulnerability.

During assessment, the psychology and inaccuracy of patient perceptions of risk and the factors leading to physicians’ underestimation of patients’ risk are significant and incompletely understood. As far as the estimation of cardiovascular risk burden is concerned, the chasm between perception and reality persists for both physicians and patients in North America, UK and the Europe.

If one restricts evidence-based cardiovascular risk reduction to statins, the question reduces to what segment of the population will be eligible for how much of what statin or polypill. Recent evidence suggests that attention to pediatric patients, at a time when habits are formed, and monitoring of adolescents as well as young adults, must increase.

The 2010 ACCF/AHA Guideline for Assessment of Cardiovascular Risk in Asymptomatic Adults, a consensus of experts, reaffirmed a central role for global risk scoring in assessing risk in all adults. Similarly, a family history was recommended for all patients.

Of all biomarkers, hs-CRP is the best studied circulating biomarker, and provides information about activation of upstream cytokines driving inflammation. High levels of hs-CRP are associated with endothelial dysfunction and predict future cardiovascular events. Use of hs-CRP is recommended or accepted in guidelines for specific patients with intermediate risk. Recent work suggests that elevations in hs-CRP levels predict a higher burden of coronary plaque, particularly mixed calcified arterial plaque, in asymptomatic individuals. Rises in hs-CRP concentrations appear to be associated with vulnerable plaque, drawing still more attention to the important role of inflammation in atherosclerosis.

In patients with high risk, aggressive treatment to reduce risk factors should be instituted early and maintained for years. Since the incubation period and signs and symptoms of atherosclerosis span decades, randomized trials of a few years’ duration provide little insight into outcomes of statin treatment over those 40–60 years. Rosuvastatin and high dose atorvastatin are drugs of choice, even if the baseline LDL-C is not elevated. Rosuvastatin produces the greatest reduction in LDL-C and improvement in apoA-I/apoB with a favorable safety profile. Lowering current LDL-C goals will undoubtedly enhance risk control and reduce event rates. However, even if all such patients received statins, cardiovascular events would still continue. The search for additional methods to lower residual risk, including using non-HDL-C as a target, raising functional HDL-C levels, and mining LDL-C subtractions for clinically useful information, continues. The recent negative trials using torcetrapib, fibrates, and extended-release niacin are significant and collectively discouraging. Nevertheless, the data reinforce the theme of this paper: intensive, unrelenting, lifestyle improvement and aggressive statin therapy are two pillars of management in the prevention of cardiovascular disease.

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