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Review

Strategies to achieve coronary arterial plaque stabilization

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Abstract

Acute coronary syndromes result from fissure, erosion or rupture of a vulnerable atherosclerotic plaque. The characteristics of a vulnerable plaque include a large lipid pool, an abundance of inflammatory cells and mediators, a reduced smooth muscle cell and collagen content and a thin overlying fibrous cap. Potential therapeutic strategies at achieving plaque stabilization have targeted these features. Lipid lowering agents, β-adrenergic blockers, angiotensin converting enzyme inhibitors and antioxidants have been shown to reduce the incidence of acute coronary syndromes, presumably through plaque stabilization. Matrix metalloproteinase inhibitors as well as macrolide antibiotics and gene therapy approaches show promise in achieving plaque stabilization. The evidence supporting plaque stabilization by these agents and the mechanisms by which these agents stabilize plaques are discussed in detail in this review.

Keywords: Atherosclerosis; Acute coronary syndromes; Plaque rupture; Lipid lowering; Matrix metalloproteinase; Hydroxymethylglutaryl-CoA reductase

1. Introduction

Acute coronary syndromes, including unstable angina, acute myocardial infarction and sudden cardiac death, result from fissure, erosion or frank rupture of a vulnerable atherosclerotic plaque [1–15]. The clinical manifestations of plaque disruption depend on the extent of thrombus formation. An acute coronary syndrome will occur only if coronary blood flow is reduced and collateral flow is inadequate [16]. Most arterial wall cracks in the coronaries are asymptomatic and do not result in arterial occlusion. Plaque ruptures can be found in 9% of all subjects and in 22% of patients with diabetes or hypertension who died of noncardiac causes [17]. It is likely that many lesions grow through plaque rupture, mural thrombus and remodeling of the plaque. Therefore, plaque stabilization may not only reduce the incidence of acute coronary syndromes but also prevent the evolution of plaques to more stenotic lesions [18].

In the majority of patients presenting with acute coronary syndromes, the culprit lesion was not significantly stenosed (<50%) on a prior recent angiogram [19–23]. This concept is supported by the demonstration of a mild residual stenosis on angiography after thrombolytic therapy for an acute myocardial infarction [22,24–26]. The severity of stenosis on coronary angiography poorly predicts the propensity of a lesion to rupture.

The intrinsic features that characterize a plaque as vulnerable are an increased lipid content, an increased macrophage, foam cell and T lymphocyte content, and a reduced collagen and smooth muscle cell content [5,6,27–32]. Rupture tends to occur at the margins or 'shoulder region' of plaques where the overlying fibrous cap is necrotic, very thin and extensively infiltrated by macrophages and adjacent to relatively normal tissue [1,4,33–35]. The 'shoulder region' is the site exposed to the greatest shear stress [36]. The extracellular lipid pool within a plaque decreases load-bearing capabilities due to poor tensile strength and results in increased stress elsewhere in the vessel wall, specifically the overlying fibrous cap [37].

The extrinsic features that cause a vulnerable plaque to rupture include increased blood pressure or vasospasm

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[23,38–40]. Acute myocardial infarction has been associated with trigger events, including emotional stress and physical activity [41–46]. Both emotional and physical stress may contribute to myocardial infarction by increasing blood pressure and by inducing coronary vasospasm. In animal models, a sudden rise in arterial pressure can produce plaque rupture in the presence of endothelial damage [1]. Despite this, most cardiac events occur without an identifiable trigger [47].

Potential therapeutic strategies to achieve plaque stabilization, resulting in a reduced incidence of acute coronary syndromes, have targeted these intrinsic or extrinsic features that promote plaque rupture. Lipid lowering agents, antioxidants, β-adrenergic blockers and angiotensin converting enzyme inhibitors have been shown to reduce the incidence of acute coronary syndromes, presumably through plaque stabilization. Strategies promoting extracellular matrix synthesis or preventing degradation within the plaque, as well as more novel gene therapy approaches, may show promise in achieving plaque stabilization.

2. Lipid lowering agents

In angiographic regression studies of lipid-lowering therapies, a small degree of plaque regression has been associated with a more substantial reduction in the incidence of clinical cardiac events (Table 1) [13,48–50]. For a review of these trials, see references [51,50] In these studies, lipid lowering therapy included hydroxymethylglutaryl coenzyme A (HMG CoA) reductase inhibitors, niacin, bile acid resins, fibrates, low density lipoprotein (LDL) apheresis, diet and exercise, and partial ileal bypass. In general, these studies have demonstrated that an improvement in the lipid profile with treatment was associated with a modest retardation of angiographic disease progression and a more marked reduction in clinical cardiac events. The two dominant hypotheses to explain the discrepancy between the magnitude of the angiographic and clinical benefit of cholesterol reduction therapy are plaque stabilization and improved endothelial function [52,53]. One or both mechanisms may play a role in the clinical benefit seen with the lipid-lowering therapies.

Elevated LDL levels have been associated with reduced endothelial function, presumably secondary to decreased nitric oxide, a potent endogenous vasodilator produced by vascular endothelial cells. A reduction in the LDL levels using lipid-lowering drugs has resulted in improved endothelial function [52,54,55]. Oxidized LDL downregulates endothelial cell nitric oxide synthase (ecNOS), the enzyme converting L-arginine to nitric oxide [56]. HMG CoA reductase inhibitors not only restore endothelial function by reducing oxidized LDL cholesterol levels, but also by directly upregulating ecNOS activity through an increase in ecNOS mRNA stability [57]. Restoration of endothelial vasodilator properties could benefit ischemia. The recovery of endothelial dysfunction and improved vasomotor function following lipid-lowering therapy could also limit vasospasm that accompanies plaque rupture, decrease recruitment of inflammatory cells and promote fibrinolysis. The improvement in endothelium-dependent vasomotor function occurs rapidly after lipid lowering, in only a matter of weeks following effective pharmacotherapy [55].

The second hypothesis to explain the discrepancy between the magnitude of the angiographic and clinical benefit of the regression trials is that lipid-lowering alters intimal plaque stability in an endothelium-independent manner. Lipids in the atheroma not only create mechanical instability, but also biologically active lipids participate in promoting oxidative stress and inflammatory responses such as monocyte migration. Lipid-lowering may therefore influence the matrix degradation cascade that appears most active in macrophage-rich areas of the atheroma, as well as promote mechanical stability within the plaque. After plaque disruption, the highly thrombogenic components of a lipid-rich core increase the subsequent risk of the formation of a potentially occlusive thrombus [58].

With regard to mechanical stability, the larger and the softer the lipid core of the plaque, the more stress the overlying fibrous cap must bear. Plaque lipid content decreases after a reduction in serum cholesterol levels in animal models [59]. Lowering of serum LDL cholesterol leads to a reduction in the amount of cholesterol entering the plaque and, more importantly, an increase in serum high density lipoprotein (HDL) cholesterol may contribute to active LDL removal from the vessel wall and from the macrophage or foam cell [60,61]. By inhibiting the oxidation of LDL, HDL may protect against excessive lipid entry into the vessel wall. Removal of lipid increases the plaque's relative collagen content and increases the production of collagen, favoring plaque stabilization [62].

Autopsy examination of the hearts of 113 men with coronary disease who had died suddenly revealed that an elevated total LDL to HDL cholesterol ratio was associated with rupture of vulnerable plaques and that a strong correlation existed between serum cholesterol and the number of vulnerable plaques in the coronary arteries [15].

Lipid lowering may stabilize plaques by reducing the expression and activity of matrix-degrading enzymes, favoring collagen accumulation in the fibrous cap, making it more resistant to rupture. In an experimental hypercholesterolemic rabbit model, reduction in serum cholesterol by cessation of the atherogenic diet resulted in a marked decrease in the macrophage-derived foam cell content within the atheroma and a resultant decrease in the macrophage-derived matrix-degrading enzymes (the matrix metalloproteinases MMP-1, MMP-2, MMP-3 and MMP-9). The decrease in matrix-degrading enzymes was associated with a substantial accumulation of interstitial collagen in the intima of the atherosclerotic lesions within the rabbits [63].

Table 1
Angiographic plaque regression studies with lipid-lowering therapies

Study	*n*	Therapy	LDL cholesterol reduction[a] (%)	Angiographic change % Change in stenosis[c] (Change in MLD in mm)		Clinical event reduction[b] (%)	Follow-up (years)
				Control	Treated		
NHLBI Type II [182]	143	Cholestyramine	26[g]	N/A (N/A)	N/A (N/A)	33	5
CLAS I [183]	188	Colestipol and niacin	43[g]	N/A (N/A)	N/A (N/A)	25	2
CLAS II [184]	138	Colestipol and niacin	40[g]	N/A (N/A)	N/A (N/A)	43	4
POSCH [185]	838	Partial ileal bypass	38[g]	N/A (N/A)	N/A (N/A)	35[g]	9.7
FATS [186]	146	Lovastatin and colestipol	46[g]	2.1% (−0.05)	−0.7%[e] (0.01)	66[f]	2.5
FATS [186]		Niacin and colestipol	32[g]	2.1% (−0.05)	−0.9%[f] (0.04)[f]	78[f]	2.5
SCOR [187]	97	Niacin and colestipol± Lovastatin	38[g]	0.8% (N/A)	−1.5%[e] (N/A)	[d]	2
STARS [188]	90	Diet alone	16[g]	5.8% (−0.23)	−1.1% (0.03)[e]	69[e]	3.25
STARS [188]	90	Diet and cholestyramine	36[g]	5.8% (−0.23)	−1.9%[f] (0.12)[g]	89[f]	3.25
Lifestyle Heart Trial [189]	48	Diet, exercise and stress management	37[f]	3.4% (N/A)	−2.2%[g] (N/A)	[d]	1
SCRIP [190]	300	Colestipol and niacin±gemfibrozil ±lovastatin	20[g]	2.8% (−0.18)	2.0% (−0.10)[e]	39[e]	4
Heidelberg [191]	113	Diet and exercise alone	8[f]	3% (−0.13)	−1%[e] (0.0)[e]	−27	1
MARS [192]	270	Lovastatin	38[g]	2.2% (0.06)	1.6% (0.03)	24	2.2
CCAIT [50]	331	Lovastatin	29[g]	2.9% (−0.09)	1.7%[e] (−0.05)[e]	22	2
MAAS [193]	381	Simvastatin	31[g]	3.6% (−0.13)	1.0%[f] (−0.04)[f]	24	4
FHRS [194]	39	LDL apheresis and simvastatin	53[e]	N/A (N/A)	−1.8% (−0.01)	[d]	2.1
FHRS [194]		Colestipol and simvastatin	44[e]	N/A (N/A)	−2.2% (0.05)	[d]	2.1
HARP [195]	79	Pravastatin±niacin ±cholestyramine ±gemfibrozil	41[g]	2.4% (−0.15)	2.1% (−0.14)	36	2.5
REGRESS [196]	885	Pravastatin	29[g]	N/A (−0.09)	N/A (−0.03)[g]	39[f]	2
PLAC I [197]	408	Pravastatin	28[g]	3.3% (−0.15)	2.1% (−0.09)[e]	60[e]	3

[a]Compared to baseline LDL prior to treatment or to control group.
[b]Clinical cardiac events were defined differently in each trial.
[c]Percent change in diameter stenosis between baseline and follow-up angiogram except in SCOR where results were reported as the percentage change in area stenosis.
[d]Too few events to calculate clinical event reduction.
[e]$p \leq 0.05$ for the comparison with the control group.
[f]$p \leq 0.01$ for the comparison with the control group.
[g]$p \leq 0.001$ for the comparison with the control group.
N/A, Not applicable or not available.
Abbreviations: MLD, minimum luminal diameter; POSCH, Program on the Surgical Control of the Hyperlipidemias; FATS, Familial Atherosclerosis Treatment Study; NHLBI Type II, National Heart, Lung and Blood Institute Type II Coronary Intervention Study; SCRIP, Stanford Coronary Risk Intervention Project; CCAIT, Canadian Coronary Atherosclerosis Intervention Trial; REGRESS, Regression Growth Evaluation Statin Study; CLAS, Cholesterol-Lowering Atherosclerosis Study; SCOR, University of California, San Francisco, Arteriosclerosis Specialized Center of Research Interventional Trial; STARS, St. Thomas Atherosclerosis Regression Study; MAAS, Multicentre Anti-Atheroma Study; FHRS, Familial Hypercholesterolaemia Regression Study; HARP, Harvard Atherosclerosis Reversibility Project; MARS, Monitored Atherosclerosis Regression Study; PLAC I, Pravastatin Limitation of Atherosclerosis in the Coronary Arteries.

Cholesterol lowering therapies may also change the proportion of the constituents of the lipid core, promoting plaque stabilization. Plaques that undergo disruption tend to be relatively soft containing a high concentration of cholesterol esters, rather than of free cholesterol monohydrate crystals [58,64–66]. In experimental cholesterol-lowering studies, cholesterol esters were converted to insoluble cholesterol monohydrate crystals, resulting in a stiffer lipid core that was more resistant to plaque rupture [66,67].

HMG CoA reductase inhibitors may also achieve plaque stabilization through effects that are independent of their cholesterol-lowering properties. Using an atherosclerotic nonhuman primate model, treatment with pravastatin as compared with an adjustable diet to maintain identical total cholesterol and HDL levels revealed no difference in the plaque size of coronary arteries by histology. However, treatment with pravastatin did demonstrate improved vasomotor function in response to acetylcholine and reduced macrophage content in the intima and media, consistent with plaque stabilization [68]. These findings were independent of the lipid-lowering effects since the diet group had the same cholesterol levels as the pravastatin group by study design. The mechanism by which macrophage content is reduced with pravastatin in this study may be related to the adhesiveness of circulating monocytes for endothelial cells of an atherosclerotic plaque. Humans with hypercholesterolemia have increased adhesiveness of isolated monocytes to fixed endothelial cells in vitro, a response that is diminished with lovastatin and simvastatin [69]. Hypercholesterolemic rats treated with fluvastatin have significantly attenuated leukocyte-adherence responses to platelet activation factor and leukotriene B_4 [69].

The larger non-angiographic clinical trials of primary and secondary prevention, including the West of Scotland Coronary Prevention Study (WOSCOPS) [70], the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) [71], the Scandinavian Simvistatin Survival Study (4S) [72] and the Cholesterol and Recurrent Events Study (CARE) [73], have confirmed the reduction in mortality and coronary events using the HMG CoA reductase inhibitors (Table 2).

3. Antioxidants

The oxidation of LDL cholesterol increases its incorporation into the arterial intima during atherogenesis. Oxidized LDL binds to the scavenger cell receptor on monocyte-derived macrophages and contributes to foam cell formation. As discussed above, oxidized LDL impairs endothelial-dependent vasodilatation and generates an inflammatory response by increasing monocyte adhesion to endothelial cells, enhancing monocyte chemotaxis, and inhibiting tissue macrophage motility. Oxidized LDL also modifies the functional response of vascular smooth muscle cells to angiotensin II stimulation, as well as promoting thrombus formation through its inhibition of the nitric oxide synthase activity of platelets and through its enhancement of tissue factor expression by monocytes [74]. Antioxidants, through their inhibition of LDL oxidation, may contribute to plaque stabilization.

Antioxidants may also promote plaque stabilization by reducing matrix degradation within the plaque. In an experimental hypercholesterolemic rabbit model, treatment with a reactive oxygen species (ROS) scavenger, *N*-acetylcysteine, markedly decreased the expression and activation of the macrophage-derived matrix-degrading enzyme MMP-9 [75]. ROSs can trigger activation of MMP precursors, which may relate to the mechanism by which *N*-acetyl-cysteine decreases MMP-9 activation [76], but the mechanism by which it affects MMP-9 expression is unclear. Treatment with other antioxidants (probucol and vitamins E and C) have been shown to reduce intimal lesions after balloon injury in hypercholesterolemic animals [77–79].

Table 2
Primary and secondary prevention studies with statins

Study	*n*	Therapy	LDL cholesterol reduction[a] (%)	Clinical event reduction[b] (%)	Follow-up (years)
WOSCOPS [70]	6595	Pravastatin	26	31[e]	4.9
AFCAPS/TexCAPS [71]	6605	Lovastatin	25[e]	37[e]	5.2
4S [72]	4444	Simvastatin	35	34[e]	5.4
CARE [73]	4159	Pravastatin	32[e]	24[d]	5.0

[a]Compared to baseline LDL prior to treatment.
[b]Clinical cardiac events were defined differently in each trial.
[c]$p \leq 0.05$ for the comparison with the control group.
[d]$p \leq 0.01$ for the comparison with the control group.
[e]$p \leq 0.001$ for the comparison with the control group.
Abbreviations: WOSCOPS, West of Scotland Coronary Prevention Study; AFCAPS/TexCAPS, Air Force/Texas Coronary Atherosclerosis Prevention Study; 4S, Scandinavian Simvastatin Survival Study; CARE, Cholesterol and Recurrent Events Study.

Numerous antioxidant vitamins have been evaluated through epidemiological studies for their association with atherosclerotic heart disease in humans, the strongest association of which has been with vitamin E (α-tocopherol). In male health professionals in the Health Professionals Follow-up Study [80] and in female nurses in the Nurse's Health Study [81] who were free of diagnosed cardiovascular disease, prospective follow-up revealed a statistically significant lower risk of clinical coronary disease among those with a higher dietary intake of vitamin E. A subgroup analysis of antioxidant vitamin intake was performed in the Cholesterol Lowering Atherosclerosis Study (CLAS), a serial angiographic clinical trial evaluating coronary artery disease progression in patients randomized to placebo or colestipol and niacin. In this analysis, subjects with a supplementary vitamin E intake of 100 IU per day or greater demonstrated less coronary artery lesion progression than did those with a supplementary vitamin E intake less than 100 IU per day (mean change in percent diameter stenosis of −0.8% vs. 2.0%, respectively with a p value of 0.04) [82]. Dietary vitamin E intake reduces LDL susceptibility to ex vivo oxidation in humans [83–86]. In experimental animals, including nonhuman primates, vitamin E reduces the formation of diet-induced atherosclerosis [87–89].

In the Cambridge Heart Antioxidant Study (CHAOS) [90], 2002 patients with angiographically proven symptomatic coronary atherosclerosis were randomized to dietary supplementation with vitamin E (400 or 800 IU) or placebo. After a median follow-up of 17 months, vitamin E treatment significantly reduced the primary composite endpoint of cardiovascular death and non-fatal myocardial infarction (MI) by 47% ($p=0.005$).

The Alpha-tocopherol Beta-carotene Cancer Prevention Study (ATBC), investigating the effects of α-tocopherol (vitamin E) and β-carotene supplements on the incidence of lung cancer in male smokers, contained a substudy evaluating the effects of these antioxidants on future coronary events in 1862 men with a prior myocardial infarction. After a median of 5.3 years of follow-up, there was no significant difference in the composite endpoint of fatal coronary heart disease and non-fatal myocardial infarction among the groups randomized to vitamin E, β-carotene, both vitamin E and β-carotene, or placebo [91].

Besides the traditional antioxidants, HMG CoA reductase inhibitors may also reduce oxidized LDL levels by increasing the total antioxidant capacity of plasma. In the Kuopio Atherosclerosis Prevention Study (KAPS) [92], pravastatin therapy for three years prolonged the lag time of LDL lipoproteins (a measure of oxidation resistance), increased plasma and LDL vitamin E levels, and improved overall LDL antioxidant capacity. Lovastatin and simvastatin have been shown to inhibit LDL oxidation and uptake by macrophages in studies of shorter duration [74].

4. β-Adrenergic blockers

Hemodynamic forces, including circumferential stress, shear stress and flexion stress, may cause disruption of a vulnerable plaque [93] According to Laplace's Law, circumferential stress is directly related to a vessel's luminal diameter and intraluminal pressure and inversely to wall thickness [94]. Consequently, the level of circumferential stress is higher in plaques with mild as compared with severe stenosis due to the larger lumen [34,93] partly explaining the fact that most acute coronary syndromes occur in plaques with mild to moderate stenoses [95]. Another explanation could be that plaques causing severe stenosis tend to have a higher fibrous and lower lipid content than those producing less severe lesions [28,96,97]. Circumferential stress is localized to the shoulder regions of plaques with soft lipid-rich cores, the most common site of rupture. Plaques repeatedly subjected to mechanical stress may eventually weaken and rupture spontaneously, a phenomenon known as 'cap fatigue' [37,58].

Triggers, including emotional stress, vigorous exercise and cold weather, may precipitate plaque rupture by a surge in sympathetic activity causing a sudden increase in blood pressure, heart rate, cardiac contractility and coronary blood flow [98]. β-Adrenergic blockers and angiotensin converting enzyme inhibitors may reduce the incidence of acute coronary syndromes by reducing the hemodynamic forces that promote plaque rupture.

Secondary prevention trials in patients with known coronary artery disease who have been treated with β-blockers have shown a reduced incidence of reinfarction and death. Meta-analysis of these secondary prevention trials has shown a 20% reduction in cardiac mortality, a 25% reduction in the incidence of reinfarction and a 30% reduction in the incidence of sudden death [99]. β-Blockade reduces the circumferential plaque stress by reducing blood pressure and blunting hypertensive pressure surges [100]. β-Blockers may also prevent plaque rupture by increasing the ability of the plaque's fibrous cap to withstand stress. The stress-bearing abilities of plaque vary with the frequency of the applied stress [101]. Because plaque stiffness increases with heart rate, β-blockers may increase plaque tensile strength by reducing heart rate [100].

5. Angiotensin converting enzyme inhibitors

The SAVE (survival and ventricular enlargement) and SOLVD (studies on left ventricular dysfunction) Treatment and Prevention Trials have demonstrated a reduction in cardiac events in patients with known coronary artery disease who have been treated with an angiotensin convert-

ing enzyme (ACE) inhibitor [102–104]. When the results of these three trials were combined, the overall risk reduction in myocardial infarction with long-term ACE inhibitor treatment was 23% [105]. The beneficial clinical effects cannot be based solely on the immediate hemodynamic effects of ACE inhibitors since the improvement in the incidence of myocardial infarction did not become apparent until after six months and broadened thereafter [106]. Other antihypertensive therapies do not provide as great a benefit as ACE inhibitors with regard to a reduction in clinical cardiac events. In a meta-analysis of 14 randomized clinical trials of antihypertensive therapies, a 5–6 mmHg reduction in diastolic blood pressure was associated with a 14% reduction in coronary heart disease events. In contrast, in SOLVD, a reduction in diastolic blood pressure of 4 mmHg yielded a 23% reduction in myocardial infarction, indicating that benefits in addition to blood pressure reduction were most probably involved [105,107]. Several mechanisms have been hypothesized to explain these results, including remodelling, anti-adrenergic effects, antiatherosclerotic effects and/or antithrombotic effects [105].

There is evidence from animal experimentation that this benefit may, in part, also be related to plaque stabilization. Infusion of angiotensin into the coronary arteries of hyperlipidemic rabbits can produce endothelial damage and can induce plaque rupture [108]. Use of ACE inhibitors in animal models of atherosclerosis has resulted not only in a decrease in the plaque area, but also a decrease in macrophage accumulation and cholesterol content and an increase in the extracellular matrix of atherosclerotic plaques, all of which promote plaque stabilization [109–112]. The ACE genotype DD, which is associated with high circulating levels of ACE, was more frequent in patients with a prior history of myocardial infarction than in control subjects [113]. Potential mechanisms for the effect of ACE inhibitors on plaque stabilization include a reduction in arterial wall stress caused by lower blood pressure or reduced levels of neurohumoral activation, or their effect on protein synthesis influencing plaque composition [114], since angiotensin II has been shown to stimulate vascular smooth muscle cell growth and proliferation [115,116].

6. Gene therapy

Gene therapy strategies aimed at stabilizing vulnerable plaques have thus far included decreasing plasma LDL and increasing plasma HDL. Transfer of the LDL receptor gene is possible for patients with familial hypercholesterolemia type IIb, a genetic deficiency in hepatic receptors for LDL cholesterol resulting in severe hypercholesterolemia and premature coronary atherosclerosis [117]. In an animal model for familial hypercholesterolemia, the Watanabe heritable hyperlipidemic (WHHL) rabbit, ex vivo transfection of hepatocytes with the LDL receptor gene and reimplantation of the transduced hepatocytes into the liver resulted in stable expression of the LDL receptor and a consistent decrease in serum cholesterol for several months [118,119]. In a pilot study of five patients with homozygous familial hypercholesterolemia who were treated with gene transfer of the LDL receptor, only two had a sustained moderate reduction in serum LDL cholesterol (~20%) [120–122]. Currently, gene therapy has limited clinical utility due to the variability in gene transfer and resultant gene expression.

Gene transfer of apo-A1, the major protein component of HDL, holds promise for achieving plaque stabilization via removal of lipid, macrophage or both. Intravenous injection of the apo-A1 gene in an adenoviral vector into mice resulted in a transient increase in serum HDL to a level comparable to that shown to be protective in humans [123]. When HDL is substantially increased therapeutically, a decrease occurs in the number and activity of macrophages and, presumably, in the occurrence of plaque rupture [60].

7. Promoters of extracellular matrix synthesis

Reduced collagen content in the fibrous cap may result from decreased synthesis of extracellular matrix by smooth muscle cells (SMCs) and/or increased breakdown by matrix-degrading proteases, thereby leading to thinning and weakening of the fibrous cap, predisposing the plaque to rupture with hemodynamic or mechanical stresses [124]. Vascular SMCs synthesize the collagenous and noncollagenous portions of the extracellular matrix. Lack of sufficient smooth muscle cells to secrete and organize the matrix in response to mechanical stress could render the fibrous cap more vulnerable to weakening by extracellular matrix degradation [18]. The extracellular matrix of plaques is primarily composed of fibrillar collagens (types I and III collagen mainly, with smaller amounts of collagen types IV, V and VI), elastin, proteoglycans and microfibrillar proteins. While the synthesis and degradation of extracellular matrix proteins are slow in the normal artery, atherosclerosis and arterial injury lead to increased synthesis of many matrix components, including elastin, collagen types I and III, and several proteoglycans. In the later stages of atherosclerosis, nonfibrillar collagen types IV and V can be found in the fibrous cap [125]. For atherosclerotic lesions causing chronic stable ischemia due to the severity of the stenosis, excess matrix accumulation is the primary mechanism of occlusion of the lumen. In contrast, unstable lesions may fail to synthesize a sufficient mass of matrix to provide strength to the fibrous cap to prevent rupture [18].

Fibrous caps that have ruptured not only have twice as many macrophages as unruptured fibrous caps but also have half as many SMCs [126]. Fewer SMCs may result in inadequate extracellular matrix production and repair. Reduced matrix synthesis may be the result of a decrease in SMC numbers secondary to inhibition of proliferation, an increase in apoptosis, or the result of inhibition of matrix gene expression in the SMCs. Cytokines and growth factors in the plaque regulate the synthesis of matrix components. Transforming growth factor-β (TGF-β) potently stimulates collagen synthesis, whereas interferon-γ (IFN-γ) suppresses the expression of collagen [127]. IFN-γ secreted by activated T lymphocytes not only inhibits collagen gene expression in SMCs but also inhibits SMC proliferation and promotes apoptosis, possibly enhanced by interleukin-1β (IL-1β)-converting enzyme (ICE) [128–133]. An inverse relation exists between the presence of T lymphocytes and interstitial collagen protein and mRNA [134]. Advanced human atheromas contain regions rich in T lymphocytes that are in a chronic state of activation, constitutively producing IFN-γ [129]. Neighboring SMCs express HLA-DR, indicating that they have been exposed to IFN-γ [135,136]. These data support the hypothesis that SMCs in the vicinity of activated T lymphocytes within the atheroma should exhibit a substantially reduced ability to synthesize interstitial collagen [134]. SMCs may therefore lack the ability to synthesize or effectively repair the surrounding extracellular matrix [137], enhancing the vulnerability of plaques to rupture. Therefore, inflammatory cells not only participate in matrix breakdown, but also inhibit matrix synthesis [133,138,139].

Apoptosis is common in atherosclerotic plaques and is virtually absent in non-atherosclerotic regions. Apoptosis occurs in SMCs subendothelially, within the fibrous cap and in the underlying media, which may destabilize the plaque and promote rupture. Apoptosis is also common in macrophages and T cells subendothelially, in the fibrous cap and in shoulder regions [132]. Subendothelially and deeper in the fibrous cap adjacent to regions with low cellularity, apoptotic SMCs are more frequent, suggesting that regions with low smooth muscle cellularity might be the result of pronounced cell reduction by apoptosis [132].

Human vascular SMCs in culture derived from atherosclerotic plaques were much more prone to cell death characteristic of apoptosis than those derived from normal arteries. The addition of insulin-like growth factor-1 (IGF-1) and platelet-derived growth factor (PDGF) markedly suppressed apoptosis of both plaque-derived and normal vascular SMCs, as did transfection with a retroviral vector containing the human *bcl*-2 gene [131].

Theoretically, inhibition of SMC apoptosis or promotion of SMC proliferation and expression of matrix gene synthesis may lead to plaque stabilization. Manipulation of cytokines and growth factors or a reduction in inflammatory cell infiltration may be potential methods to achieve these goals. Unfortunately, enhancement of matrix synthesis may lead to increased plaque growth, resulting in more severe stenosis and arterial occlusion and lead to more frequent restenosis after percutaneous coronary intervention.

8. Inhibitors of plaque inflammation and extracellular matrix degradation

Increased matrix degrading activity associated with vascular smooth muscle cells, macrophages and T lymphocytes is a common finding in unstable atherosclerotic lesions and could lead to weakening of the fibrous cap and resultant plaque rupture. There are three major pathways of extracellular matrix degradation: the serine proteases, the cysteine proteases and the matrix metalloproteinases [18]. The vast majority of evidence suggesting that matrix degradation may be involved in plaque rupture has focused on the matrix metalloproteinases (MMPs). Plaque stabilization could be achieved through the inhibition of extracellular matrix degradation either by preventing the accumulation of macrophages and T lymphocytes in the atherosclerotic plaque or by inhibiting the proteolytic enzyme cascade directly.

The matrix metalloproteinase superfamily (Table 3) includes three main classes: (a) the collagenases, which are enzymes specialized in the initial cleavage of tightly coiled, native triple helical collagen that confer strength to the fibrous cap, (b) the gelatinases, which can catalyze the further breakdown of collagen fragments and (c) the stromelysins, which can activate other members of the MMP family and can degrade a broad spectrum of matrix constituents, including proteoglycan core proteins and elastin. A new group of MMPs, membrane-type MMPs (MT-MMPs), has recently been identified. Instead of being soluble, MT-MMPs contain a transmembrane domain, thus attaching to the surface of the cell [140]. With the exception of the MT-MMPs, the MMPs are secreted as inactive proenzymes or zymogens that must be activated by cleavage of the N-terminus. Endogenous inhibitors, called tissue inhibitors of metalloproteinases (TIMPs), bind to the active sites of MMPs and regulate their activity [141,142].

Multiple histologic studies have demonstrated that MMP expression by vascular smooth muscle cells and macrophages is increased in human atherosclerotic plaques (Fig. 1) [143]. Increased stromelysin (MMP-3), interstitial collagenase (MMP-1) and gelatinase expression was noted in human coronary atherosclerotic plaques [144]. Interstitial collagenase expression was found at the borders of lipid cores and in subsets of smooth muscle cells and endothelial cells in human carotid atherosclerotic plaques [145]. Interstitial collagenase colocalized with regions of circumferential tensile stress in the fibrous cap of the atherosclerotic plaque, which may play a role in the pathogenesis

Table 3
The matrix metalloproteinase superfamily

Nomenclature	Substrates
Collagenases	
Interstitial collagenase (MMP-1)	Collagen types I, II, III (III>>I), VI and X, gelatin, proteoglycan
Neutrophil collagenase (MMP-8)	Same as interstitial collagenase (I>>III)
Collagenase-3 (MMP-13)	
Gelatinases	
Gelatinase A (MMP-2)	Gelatins, collagen types IV, V, VII, X and XI, elastin, fibronectin, proteoglycan
Gelatinase B (MMP-9)	Gelatins, collagen types IV and V, elastin, proteoglycan
Stromelysins	
Stromelysin (MMP-3)	Proteoglycan, fibronectin, laminin, elastin, gelatin, collagen types II, IV, V, IX and X
Stromelysin-2 (MMP-10)	Same as stromelysin
Stromelysin-3 (MMP-11)	Gelatin, fibronectin, proteoglycan
Matrilysin (MMP-7)	Gelatin, fibronectin, laminin, collagen type IV, proteoglycan
Metalloelastase (MMP-12)	Elastin
Membrane-type MMPs	Collagen type IV, gelatin
MT-MMP-1 (MMP-14)	
MT-MMP-2 (MMP-15)	
MT-MMP-3 (MMP-16)	
MT-MMP-4 (MMP-17)	
Unclassified	
MMP-18	
MMP-19	

Adapted from Ye et al. [198] with permission.

of plaque rupture. The increased expression of MMP may represent an adaptive response to mechanical stress, allowing the fibrous cap to remodel to a more structurally sound configuration, but this remodeling may transiently allow the plaque to become vulnerable to rupture [146–148]. Matrilysin (MMP-7) and metalloelastase (MMP-12) were expressed in lipid-laden macrophages within human atherosclerotic lesions in carotid endarterectomy samples but not in normal arteries [149]. The presence of gelatinase B (MMP-9) was noted in coronary atherectomy specimens from patients with acute ischemic syndromes with recent plaque rupture (unstable angina) and stable angina, but not in normal internal mammary arteries. Intracellular localization of gelatinase B, indicative of active synthesis, was much more prevalent in the specimens from patients with unstable angina than from those with stable angina [150].

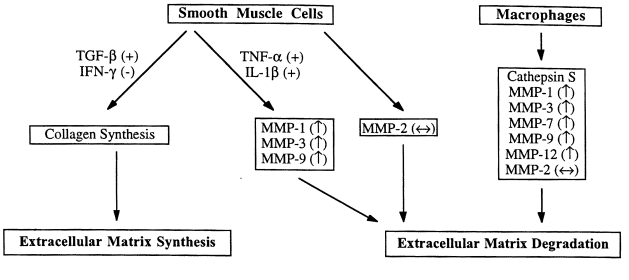

Fig. 1. Simplified diagram of extracellular matrix synthesis and degradation by smooth muscle cells and macrophages within the atherosclerotic plaque, including the cytokines influencing this process. ↑ indicates increased expression and ↔ indicates similar expression in the atherosclerotic plaque compared to the normal vessel. + indicates activation and − indicates inhibition of the process by the cytokine indicated. See text for details and abbreviations.

The association of activated T lymphocytes and macrophages with plaque rupture was made after histologic examination of the culprit lesions in 20 patients who died of an acute myocardial infarction. T lymphocytes and macrophages predominated at the sites of plaque disruption (deep intimal rupture or superficial erosions). Adjacent SMCs, macrophages and lymphocytes expressed high levels of HLA-DR, an indicator of an active inflammatory reaction, which contrasted markedly with the low or absent expression of HLA-DR elsewhere in the fibrous cap [30]. Only IFN-γ secreted by activated T lymphocytes induces the expression of HLA-DR, suggesting the presence of this cytokine at the sites of plaque rupture in humans [135]. Inflammation plays a key role in destabilizing the fibrous cap tissue and causing plaque rupture.

Increased macrophage density and/or activation in the atherosclerotic plaque may induce collagen breakdown in the fibrous cap by secreting MMPs, thus contributing to the vulnerability to plaque rupture. Coronary plaques removed during directional atherectomy in patients with acute coronary syndromes (unstable angina and non-Q-

wave myocardial infarctions) were shown to have at least a fourfold greater macrophage infiltration compared with plaques of patients with stable angina [151]. Increased macrophage density and reduced collagen content have been shown by in vitro mechanical testing to be associated with a reduced tensile strength [152]. When fibrous caps of human atherosclerotic plaques were exposed to human monocyte-derived macrophages in cell culture, there was biochemical evidence of increased collagen degradation as compared to cell-free culture medium, and this degradation was inhibited in the presence of an MMP inhibitor [124].

Vascular smooth muscle cells in culture constitutively express gelatinase A (MMP-2), which degrades nonfibrillar collagen and participates in cellular migration, as well as TIMPs [153,154]. In the presence of cytokines, such as TNF-α or IL-1β, cultured human vascular smooth muscle cells markedly increase the synthesis of interstitial collagenase (MMP-1), stromelysin (MMP-3) and gelatinase B (MMP-9) [147,153]. Taken together, the MMPs produced by vascular SMCs in culture can digest all the main components of the arterial wall extracellular matrix. Like unstimulated SMCs in culture, SMCs of normal arterial tissue express MMP-2 and TIMPs. In contrast, SMCs of the atherosclerotic plaque, as well as cultured stimulated SMCs, express, in addition, MMP-1, MMP-3 and MMP-9 [147].

Reduction in macrophage and T lymphocyte infiltration and the resultant reduction in protease and cytokine release may help stabilize plaques by decreasing extracellular matrix degradation and strengthening the fibrous cap. Lipid-lowering and antioxidants through their reduction in oxidized LDL have been shown to reduce macrophage infiltration in experimental models [155–159]. If a plaque's macrophage content is reduced, then the secretion of collagen-degrading proteases will be reduced and, theoretically, may result in plaque stabilization.

MMP inhibition also represents a potential therapeutic strategy aimed at stabilizing plaques by reducing extracellular matrix degradation. Possible methods to achieve MMP inhibition include (i) increasing the levels of natural inhibitors (TIMPs) either by exogenous administration of recombinant TIMPs or by an elevation in their local production, (ii) administering synthetic inhibitors, or (iii) decreasing MMP production (Fig. 2). TIMP-1 is synthesized by most types of connective tissue cells, as well as macrophages, and inhibits all members of the collagenase, stromelysin and gelatinase classes of MMPs. It forms high-affinity, irreversible, noncovalent complexes with the active form of the enzymes. TIMP-2 can also inhibit all members of the collagenase, stromelysin and gelatinase classes of MMPs, especially gelatinase A. TIMP-1 is highly inducible by cytokines and hormones, whereas TIMP-2 expression is largely constitutive, following the pattern of expression of gelatinase A [141].

Exogenously administered TIMPs are readily metabolized and denatured and tend to aggregate and adhere to surfaces, thereby limiting tissue penetration. They have been used in mouse models of arthritis without convincing demonstration of effectiveness. The upregulation of TIMP production may be achieved with the administration of cytokines, such as TGF-β or retinoids, but it is likely that these compounds will have numerous side effects limiting their use, since they are active in many cellular processes. Continued improvement in transfection techniques makes a molecular approach to overexpressing the TIMP-1 or TIMP-2 genes more attractive, as demonstrated in nude mice with reduction in local neoplastic invasion [141].

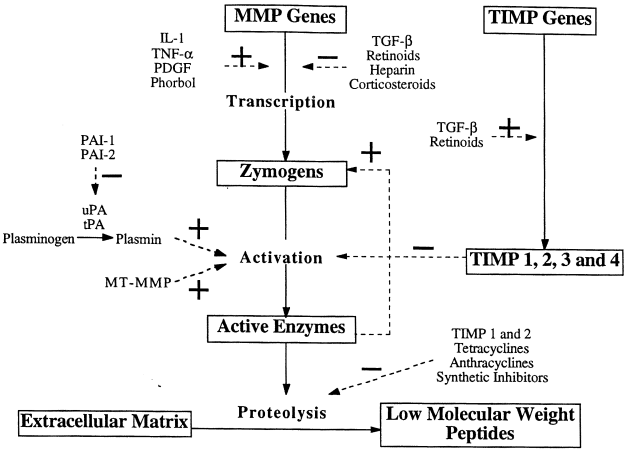

Fig. 2. Transcription and activation of the matrix metalloproteinases including the negative (−) and positive (+) factors influencing this process. See text for details and abbreviations. Adapted from Dollery et al. [141] with permission.

Synthetic inhibitors of MMPs, including tetracycline-derived antibiotics, anthracyclines and synthetic peptides, have been unimpressive in animal models and in clinical trials for rheumatoid arthritis. Retinoids and corticosteroids downregulate MMP transcription, however, they have proven ineffective in the treatment of restenosis after coronary angioplasty [141]. Moreover, not all of the matrix-degrading enzymes in the plaque are MMPs, and the contribution of these non-MMPs to plaque destabilization requires consideration. The long-term effects of MMP inhibitor use also requires evaluation since MMPs are involved in many physiologic processes, including arterial remodelling, wound healing and angiogenesis.

Although MMPs can be activated by many factors in vitro, the fibrinolytic pathway, specifically plasmin, may be one of the critical mediators for extracellular activation of latent MMPs to their active form in vivo. Macrophage- and SMC-derived uPA and tPA, by acting on its cell surface receptor, can thus generate plasmin near the cell surface, thereby facilitating activation of latent MMPs near the cell surface, where they are less susceptible to the action of TIMP [160–162]. Local activation of the plasminogen activator system not only leads to enhanced

extracellular membrane (ECM) degradation but also to activation of TGF-β, a powerful inducer of PAI-1 biosynthesis in endothelial cells and SMCs, resulting in a decrease in PA activity. As mentioned previously, TGF-β also induces biosynthesis of extracellular matrix components. TGF-β or inhibitors of the plasminogen activators may represent a possible therapy aimed at achieving plaque stabilization [162].

The CD40 pathway represents a possible signaling mechanism through which macrophages and vascular SMCs in atheroma can be induced to express matrix-degrading proteinases, resulting in plaque rupture. The CD40 receptor, a member of the TNF receptor family, is located on the surface of SMCs and macrophages, and activated T lymphocytes can express CD40 ligand, a TNF-like molecule, on their cell surface [163]. Human monocytes and macrophages in culture were stimulated through the CD40 receptor by either membranes from activated T lymphocytes or by recombinant CD40 ligand, resulting in increased expression of interstitial collagenase and stromelysin as well as tissue factor protein and activity that initiates thrombosis [164]. The addition of IFN-γ or antibody to CD40 ligand inhibited the expression of these MMPs by macrophages [164]. Stimulated human T lymphocytes also induced the expression of interstitial collagenase, stromelysin, gelatinase B and activated gelatinase A in human vascular SMCs in culture via CD40 ligation. Recombinant human CD40 ligand induced the synthesis of interstitial collagenase, stromelysin and gelatinase B on vascular SMCs in culture and stimulated the expression of these enzymes to a greater extent than did maximally effective concentrations of TNF-α and IL-1β. IFN-γ or neutralizing antibody to CD40 ligand inhibited the induction of MMPs by CD40 ligand in SMCs [165]. Interruption of CD40 signaling could provide a novel means of plaque stabilization by preventing MMP-induced matrix degradation.

Systemic evidence of acute inflammation, manifested by elevations in C-reactive protein (CRP) and serum amyloid A protein (SAA), is more prevalent in patients with unstable angina or acute myocardial infarction compared to those with stable angina and represents an independent predictor of worse outcome with higher rates of death, MI and myocardial revascularization [166–171]. The elevation in acute phase reactants may reflect intrinsic inflammation and tissue injury within the atherosclerotic plaque or may reflect inflammation or tissue injury elsewhere in the body that promotes atherogenesis and plaque instability [168]. Ischemia-induced endothelial damage, oxidized LDL, immune complexes and infection are all potential causes of vascular injury and an acute-phase response [167]. For example, *Helicobacter pylori*, *Chlamydia pneumoniae* and cytomegalovirus (CMV) infections are associated with increased CRP and possibly coronary heart disease. CRP also stimulates the production of tissue factor by monocytes and macrophages, predisposing to thrombosis if plaque rupture or erosion should occur [168].

9. Macrolide antibiotics

Epidemiologic data has suggested the association between infectious agents and coronary heart disease, especially with *Helicobacter pylori*, *Chlamydia pneumoniae* and CMV [172,173]. Potential causative mechanisms include both direct effects of the infectious agent on the arterial wall (endothelial injury or dysfunction, SMC proliferation and local inflammation) and indirect effects mediated in the circulation through chronic inflammation, cross-reactive antibodies, or alterations in cardiovascular risk factors (lipids, coagulation proteins, oxidative metabolites or homocysteine) [173].

The strongest association between an infectious agent and coronary heart disease has been with *Chlamydia pneumoniae*. Epidemiological studies demonstrate a consistent association between elevated *Chlamydia pneumoniae* antibody titres and myocardial infarction and coronary heart disease [174–177]. *Chlamydia pneumoniae* elementary bodies and DNA have been found within atheromatous coronary arteries [178,179]. Since laboratory culture of this intracellular pathogen is difficult, elevations in antibody titre may reflect new, reactivated or remote infection.

The predictability of *Chlamydia pneumoniae* antibody titres on future cardiovascular events and the effect of azithromycin (an antichlamydial macrolide antibiotic) on future cardiovascular events were evaluated in 213 male survivors of myocardial infarction. Fifty-nine patients were seronegative, 74 had intermediate titres and 80 were strongly seropositive. Patients with strong persistent seropositivity were randomized to either a short course of oral azithromycin therapy or placebo. Over a mean follow-up period of one and a half years, the incidence of cardiovascular events (defined as nonfatal MI, cardiovascular death, unstable angina requiring either intravenous anti-anginal therapy, coronary angioplasty or urgent coronary bypass surgery) increased with increasing *Chlamydia pneumoniae* antibody titre. Treatment with azithromycin in the seropositive patients reduced the incidence of cardiovascular events fivefold, reaching the risk of those seronegative patients. Patients receiving azithromycin therapy were more likely to experience a reduction in IgG *Chlamydia pneumoniae* antibody titres than those in the placebo group. The authors concluded that an increased *Chlamydia pneumoniae* antibody titre in post-MI patients may be a predictor for further adverse cardiovascular events, and administration of a short course of azithromycin in the seropositive patients may lower this risk, possibly by acting against *Chlamydia pneumoniae*. Azithromycin, by suppressing or eradicating infection, may

have helped to stabilize active plaque lesions, in part by reducing inflammation and hypercoagulation [180].

Another randomized trial, the ROXIS pilot study, assigned 202 patients with unstable angina or non-Q-wave myocardial infarction to roxithromycin (an antichlamydial macrolide antibiotic) or placebo for 30 days. Almost half of the patients in each group were seropositive for *Chlamydia pneumoniae*. A statistically significant 78% reduction in the 30 day composite endpoint of cardiac death, myocardial infarction and recurrent ischemia was observed in the roxithromycin-treated group [181].

10. Conclusion

Using the occurrence of an acute coronary syndrome as a surrogate marker for plaque rupture, indirect evidence exists to suggest that plaque stabilization can be achieved through lipid-lowering, β-adrenergic blockade and ACE inhibition. The characteristic appearance of plaque rupture on angiography, angioscopy or intravascular ultrasound provides direct evidence of plaque rupture but is a rather insensitive marker to adequately assess the plaque-stabilizing effects of therapies in human clinical trials. Unfortunately, no animal model has yet been developed to mimic atherosclerotic plaque rupture. Theoretically, antioxidants, inhibition of matrix metalloproteinases and reduction in inflammatory cell infiltration into atherosclerotic plaques hold promise for achieving plaque stabilization.

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