The Role of Collagenases in the Pathogenesis of Vulnerable Atherosclerotic Plaques

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Abstract: Acute coronary syndromes including acute myocardial infarction, unstable angina, and cardiac sudden death are often triggered by rupture of the vulnerable atherosclerotic plaque. Among various extracellular matrices, interstitial collagen plays a key role in the mechanical strength of the artery. Members of the matrix metalloproteinase (MMP) family produced by macrophages, in particular interstitial collagenases (MMP-1/collagenase-1, MMP-8/collagenase-2, MMP-13/collagenase-3), likely contribute to plaque vulnerability. Recent clinical trials have demonstrated that lipid lowering with statins reduces the onset of the acute coronary syndromes in patients despite only modest reduction in stenoses detected by angiography, thus suggesting the possibility that lipid lowering prevents coronary events mainly by reducing plaque vulnerability (“stabilization”) rather than merely shrinking the plaque burden (“regression”). Preclinical studies including our own support this concept of plaque stabilization. We have shown in hypercholesterolemic rabbits that lipid lowering by either diet or statin treatment reduces a number of features typical of the “vulnerable” plaque: oxidative stress, endothelial cell dysfunction, smooth muscle cell activation, and macrophage infiltration. An inverse relationship between reduced MMP-1/collagenase-1 and increased collagen in the intima of rabbit aorta suggests the important role of collagenase action in collagen metabolism in atheroma. However, further studies are needed to address direct in vivo evidence that links action of the MMP family collagenases with regulation of the collagen content of atheroma, critical to the clinical complications of coronary atherosclerosis.

Key words: atherosclerosis, coronary disease, hypercholesterolemia, inflammation, metalloproteinases

Vascular Inflammation and Plaque Vulnerability

Disruption of the atheromatous plaque participates in the pathogenesis of thrombus formation and consequent acute coronary syndromes such as unstable angina, acute myocardial infarction, or cardiac sudden death. Inflammation plays a fundamental role in the pathogenesis of atherosclerosis and triggers the onset of acute coronary events. Pathological studies have demonstrated that rupture-prone lesions usually contain a prominent accumulation of inflammatory cells including macrophages underlying a thin and collagen-poor fibrous cap. (Table 1) Activated endothelial cells expressing inflammatory mediators (cell adhesion molecules and chemokines) recruit leukocytes into the arterial wall and play a critical role in the formation of macrophage-rich atheroma. Extracellular matrix macromolecules, notably interstitial collagens, confer strength on the plaque's fibrous cap. Fibroblast expression of enzymes including matrix metalloproteinases (MMPs) in atheroma may cause loss of collagen in the plaque's protective fibrous cap and promote disruption, which causes the direct contact of blood coagulation factors to tissue factor, a potent activator of coagulation cascade overexpressed by lesional macrophages, and may in turn accelerate thrombus formation. This article will discuss the potential role of inflammation and collagenolytic activity in the breakdown of collagen in atheroma and therapeutic approaches for this disease process.

Imbalance of Collagen Production and Degradation in Atheroma

Intimal SMCs in atherosclerotic plaques show features distinctive from SMCs in the apparently normal artery or the tunica media. These features include apoptosis, a form of programmed cell death. Smooth muscle cells (SMCs) are a major source of collagen production in atheroma, and reduction of this cell type due to apoptosis likely causes loss of collagen in the atheroma's fibrous cap. Accumulation of T lymphocytes in the fibrous cap is another feature typical of atheroma prone to rupture. Interferon-γ (IFN-γ), a product of activated T cells, may contribute to collagen loss in atheroma. Amento et al. demonstrated that IFN-
y reduces synthesis of collagen I and III by human SMCs stimulated by other cytokines or growth factors including interleukin-1 (IL-1), platelet-derived growth factor (PDGF), or transforming growth factor-β (TGF-β).

In addition to decreased collagen production, increased proteolytic activity may promote plaque vulnerability. A number of studies including our own have demonstrated the expression and activity of MMPs in experimental and human atherosclerotic lesions. In particular, collagenases of the MMP family, including MMP-1/collagenase-1, MMP-8/collagenase-2, and MMP-13/collagenase-3, break down fibrillar collagens, key determinants of the tensile strength of plaques. Interstitial collagen types I and III are major arterial collagens in human atherosclerotic plaques. MMP family collagenases initiate degradation of type I collagen at specific cleavage sites between Gly775 and Ilc76 of the α1(I) chain. Other members of the MMP family, such as MMP-2 and MMP-9, further cleave degraded collagen fragments. Macrophages in atherosclerotic plaques express all MMP family collagenases (MMP-1/collagenase-1, MMP-8/collagenase-2, and MMP-13/collagenase-3), suggesting their potential role in the pathogenesis of collagen breakdown and plaque vulnerability.

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<thead>
<tr>
<th>Table 1. Pathogenesis of plaque, vulnerability.</th>
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<td>Loss of smooth muscle cells due to apoptosis</td>
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<td>Impaired collagen synthesis by smooth muscle cells</td>
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<td>Inflammatory cell accumulation (macrophages, T lymphocytes)</td>
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<td>Cleavage of interstitial collagen by collagenases (MMP-1, MMP-8, MMP-13)</td>
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<td>Degradation of cleaved collagen by other MMPs (MMP-2, MMP-9, etc.)</td>
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<td>Oxidative stress</td>
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<td>Endothelial activation/dysfunction</td>
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Stabilization of the Atherosclerotic Plaque by Lipid Lowering

For several decades in the last century, a number of preclinical studies addressed the hypothesis that atherosclerotic lesions can be regressed. However, recent clinical trials have suggested that lipid lowering therapy with HMG-CoA reductase inhibitors (statins) effectively prevents onset of the acute coronary syndromes despite the modest reduction in stenoses determined by angiography. This discrepancy suggested the possibility that lipid lowering reduces acute coronary events by modifying the nature of vulnerable plaques functionally ("stabilization") rather than by reducing the lesion size ("regression").

Several lines of evidence from preclinical studies including our own support the hypothesis of plaque stabilization. We have demonstrated in a rabbit model of atherosclerosis that lipid lowering by diet improves a number of features associated with vulnerable plaques in human coronary arteries such as vascular inflammation, proteolytic activity, and thrombogenicity. Dietary lipid lowering improves endothelial functions and oxidative stress in rabbit atheroma (i.e., reduced expression of cell adhesion molecules and production of reactive oxygen species) that should limit macrophage accumulation. Aortic atheroma of hypercholesterolemic rabbits contained various MMPs including MMP-1/collagenase-1, that resemble vulnerable plaques in the human coronary artery. However, dietary lipid lowering reduced macrophages expressing MMP-1/collagenase-1 and concomitantly increased interstitial collagen in ath- eroma, an inverse relationship that not only suggests a potential mechanism of plaque stabilization by lipid lowering but also provides indirect in vivo evidence that collagenases play an important role of collagenases in collagen metabolism in atheroma. We also reported that lipid lowering favors the accumulation of SMCs that exhibit a more mature phenotype and express less MMPs in the fibrous cap of rabbit atheroma compared to those from the baseline lesions. Kockx et al. also reported similar results on the effects of dietary lipid lowering on macrophage accumulation and collagen content in rabbit atheroma. Despite a limited number of subjects, a study of pravastatin by Crisby et al. demonstrates decreased MMP expression and increased collagen content in human carotid plaques by lipid lowering, and also supports the preclinical studies discussed above.

More recently, we have reported that lipid lowering with statins also reduced macrophage-derived MMPs in rabbit atheroma. Statins inhibit HMG-CoA reductase, a rate-limiting enzyme in the cholesterol synthesis pathway. Inhibition of HMG-CoA reductase reduces not only the cholesterol biosynthesis but also production of other intermediate metabolites that critically modify protein functions. Although little doubt remains regarding the importance of lipid lowering itself as discussed above, accumulating preclinical and clinical evidence also suggests that statins have lipid-independent effects on vascular inflammation and plaque vulnerability. Statin treatment reduces macrophage growth in vitro. Statins also reduce macrophage expression of MMPs in vitro. However, it should be noted that most in vitro studies demonstrating so-called pleiotropic effects of statins have employed concentrations on the order of 1 to 100 μM. Such high levels of statins exceed those achieved in plasma or tissue during clinical therapy. Also, potential direct effects on vascular cell functions may vary among statins as we have shown that hydrophilic and lipophilic statins have differential effects on collagen accumulation in rabbit atheroma.

The Role of MMP Family Collagenases in Collagen Breakdown in Atheroma

This article has thus far discussed that accumulating indirect evidence suggests a role for MMP family collagenases in the pathogenesis of plaque disruption. Because we lack direct in vivo evidence that establishes that collagenolysis actually regulates the interstitial collagen content of atheroma, the readers may ask whether MMPs are really key players in plaque disruption.
Fig. 1. An inverse relationship between MMP-1/collagenase-1 expression and interstitial collagen content in atheroma of hypercholesterolemic rabbits.

The rabbit aortic lesion after 4 month on a high-cholesterol diet (Baseline lesion) contained many macrophages expressing high level of MMP-1/collagenase-1. Picrosirius red staining with polarization barely detected accumulation of interstitial collagen on a serial section. 16 months of dietary lipid lowering decreased MMP-1/collagenase-1 expression and in parallel increased collagen content, a key determinant of plaque stability. Original magnification: ×10. From Aikawa, M., et al., Ref. 16), with permission.

Sukhova et al. from our group have demonstrated that human atheroma contain elastinolytic cathepsins K and S. Cathepsin K is also known as a collagenase even more potent than MMP family collagenases. The relative contribution of MMP family collagenases and other collagenases such as cathepsin K to collagen breakdown in atheroma has not been determined as yet. Lemaitre et al. have demonstrated that macrophage-selective overexpression of human MMP-1/collagenase-1 in apolipoprotein E (apoE)-deficient mice decreases lesion size, and further concluded that matrix remodeling by MMP-1/collagenase-1 is beneficial in the progression of atherosclerosis. However, their study did not provide quantitative analysis of collagen accumulation in atheroma.

We recently explored direct evidence in vivo for the role of collagenases in the collagen metabolism, employ-
ing “knock-in” mice that bear a mutation, introduced by homologous recombination, at the collagenase cleavage site of type I collagen [α1(I) chain] (Ile726 to Pro726). We introduced this mutation into hypercholesterolemic apoE-deficient mice and observed that collagenase-resistance produced a statistically significant increase in the intimal collagen content (original manuscript submitted). These results provide unambiguous in vivo evidence for a role of MMP family collagenases in collagen breakdown.

CONCLUSIONS

Recent advances in vascular biology provide new insights into the mechanisms by which hypercholesterolemia induces vulnerability in atherosclerotic plaques prone to coronary thrombosis. Because lipid lowering in experimental animals actually improves a number of features associated with plaque vulnerability, we suggested that lipid lowering plays an important role independent of the “pleiotropic” effects of statins. Such preclinical studies substantially increased our mechanistic understanding of plaque stabilization. However, many issues remain unresolved. For example, the majority of acute coronary syndromes still occur in patients who have undergone aggressive, long-term lipid lowering therapy with statins, thus necessitating the establishment of other therapeutic approaches beyond lipid lowering. Studies employing new technologies are also needed to further explore new mechanisms of vascular inflammation and plaque vulnerability.

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