Atherosclerosis continues to cause considerable morbidity and mortality in the Western countries. In response to cardiovascular risk factors, a cascade of compensatory structural events occurs within the vessel wall. It has become increasingly evident that significant alterations in the structure and composition of the extracellular matrix (ECM) play a key role in the atherogenic process. Far from being a static structure, ECM is a dynamic interactive milieu undergoing continuous remodeling that critically influences cell functions. The activity of proteolytic enzymes is the rate-limiting step in ECM degradation. It is now well established that among numerous other proteinases, the matrix metalloproteinases (MMPs) play the major role in the degradation of collagen and other ECM components. Expressed at low levels in normal adult tissue, MMPs are upregulated in physiological and pathological remodeling processes. MMPs are specialized enzymes involved in processes such as wound healing, but there is growing interest focusing on a pathological role for MMPs in vascular disease states.

MMPs are a family of zinc-dependent proteases produced by a variety of cell types, including endothelial, smooth muscle cells (SMC), and monocytes. They are classified into subgroups based upon substrate specificity and/or structure, including collagenases (MMP-1,-8,-13,-18), stromelysins (MMP-3,-10,-11), gelatinases (MMP-2,-9), and the membrane-type MMPs (MT-MMPs). MMP activity is tightly regulated at both intracellular and extracellular levels. Growth factors, cytokines, hormones, and tumor promoters regulate MMP expression at the transcriptional level, while heparin, transforming growth factor-β (TGF-β), and corticosteroids have an inhibitory effect. Extracellular activation of the latent proenzymes represents a second level of control. The major physiological activator of MMPs is plasmin, which activates pro-MMPs to active MMPs (Figure). A further level of control of MMP activity involves the binding of MMPs to specific tissue inhibitor of metalloproteinases (TIMPs). At least four members of TIMP gene family are known: TIMP-1,-2,-3, and -4, all sharing structural features. TIMPs inhibit MMPs by binding irreversibly to the active form of the enzyme. Overall, proteolytic activity depends on the relative concentration of the active enzymes and their inhibitors.

**Role of MMPs in Atherosclerosis**

Alterations in ECM homeostasis, due to changes in the synthesis and/or degradation of the arterial ECM, have been associated with vascular diseases. There is growing evidence that MMPs are involved in all stages of the atherosclerosis process, from the initial lesion to plaque rupture.

Plaque formation occurs as a result of cellular migration and proliferation accompanied by an accumulation of ECM. MMP-2-dependent vascular ECM degradation promotes SMC migration and early plaque development. During the initial period of development of atherosclerotic plaque, outward growth produces compensatory enlargement that involves matrix remodeling. Decreased arterial compliance in hypertension and aging correlates with accumulation of collagen and loss
of elastin. In the latter stages of atherosclerosis, thrombotic complications often result from disruption of the atherosclerotic plaque due to rupture of the fibrous cap or superficial erosion of the endothelium. Both of these processes largely depend on excessive ECM breakdown. Neovascularization within the plaque may also play a role in promoting plaque destabilization. Intraplaque angiogenesis is influenced by MMP activity through interactions between integrins and proteinases. Finally, aneurysm formation represents an extreme stage of arterial remodeling due to increased ECM breakdown mediated by MMP-2 and-9.

Immunocytochemistry, zymography, and in situ hybridization studies have demonstrated an increased expression of different MMPs in human atherosclerotic plaques. More recent work has shown an increase of MMP-9 in unstable carotid plaques based on symptomatology, spontaneous embolization, and histological evidence of instability. Furthermore, a significant increase in circulating MMP-9 levels was observed in patients undergoing carotid endarterectomy with evidence of ongoing spontaneous embolization.

The messenger molecules that mediate communication between the various cell types involved in vascular proteolysis in atherosclerosis are poorly understood. However, there has been considerable interest in the role of the CD40/CD40L system. Ligation of CD40 on atheroma-associated cells causes activation of MMPs and enhanced production of cellular adhesion molecules and proinflammatory cytokines, chemokines, and tissue factor, and thus leads to progression of atherosclerosis, plaque destabilization, and the formation of intravascular thrombus.

MMPs as Therapeutic Targets in Atherosclerosis

Because of the relevance of MMPs in atherosclerosis, it is not surprising that MMP inhibition represents a potential therapeutic strategy aimed at stabilizing plaques by reducing ECM degradation and restoring the MMP/TIMP equilibrium. Potential methods for MMP inhibition include increasing TIMP levels, administration of inhibitors of MMP activity, and reducing MMP production.

TIMP levels could be increased by the exogenous administration of recombinant TIMP or stimulating increased expression throughout gene therapy. A number of synthetic inhibitors have been investigated, in particular specific synthetic peptides, tetracycline-based antibiotics, and anthracyclines with promising results in limiting aneurysm expansion in experimental animal models.

We had previously shown that hypercholesterolemia induces several structural and morphologic changes in the vessel wall of pigs after angioplasty. More recently, we found that a cholesterol-rich diet increased vascular MMP-1 expression after injury in this porcine model of atherosclerosis. Interestingly, we have demonstrated that antioxidant vitamins C and E, when given in combination, markedly reduced the hypercholesterolemia-induced changes in vascular and the systemic expression of collagenase (MMP-1), while increasing the collagen content, thus providing additional evidence of the role of MMP-1 in plaque stabilization and the beneficial effect of antioxidant vitamins on the severity of atherosclerosis in this experimental model. The mechanisms by which antioxidants may participate in matrix-protective processes remain unclear. Oxidative stress is increasingly being recognized as a potentially important contributor to atherogenesis and restenosis after vascular injury. NF-κB, a transcription factor activated by oxidative stress has been shown to be required for cytokine up-regulation of MMP-1 in vascular cells. The fact that dietary supplementation with vitamins C and E reduced plasma lipid peroxidation in the
porcine model would support this hypothesis. Whereas controversy still exists as to the role of antioxidants in the prevention of cardiovascular disease in humans, the beneficial effects of lipid-lowering agents, mainly statins in coronary and cerebrovascular disease has been consistently reported. Independently of their lipid-lowering effects, statins have been shown to reduce vascular MMPs expression, as well the inflammatory response, in vascular disease (pleiotropic effects).

In conclusion, there are convincing data suggesting that an imbalance between MMP levels and their inhibitors may shift the equilibrium from matrix accumulation towards matrix degradation, leading to plaque disruption that precedes the onset of acute occlusive vascular syndromes. Thus, MMPs represent an attractive target to prevent plaque destabilization. Different therapies, including antioxidant vitamins and statins, can contribute to prevent matrix degradation in atherosclerosis.

References


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Figure. Proposed mechanisms of MMPs in atherothrombosis

INACTIVE PRO-MMPS

Thrombin Plasmin MT-MMP

Autocatalysis

ACTIVE MMPs

CD40/CD40L

TIMPs

ECM DEGRADATION

Cell migration/proliferation Plaque rupture Neovascularization

ATHEROTHROMBOSIS