Reactive Oxygen Species, Metalloproteinases, and Plaque Stability

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Understanding of the factors that lead to atherosclerotic plaque instability causing thrombosis is increasing rapidly. The morphological characteristics of plaques that are unstable, ie, complicated by recent thrombosis, lend insight into the structural and cellular features of presently stable plaques that are vulnerable, ie, at high risk of becoming unstable in the future. The risk of any individual with coronary atherosclerosis developing an acute ischemic event depends on the number of such vulnerable plaques present in that individual rather than the number of plaques overall. One factor in the variation in risk for a further acute event after, for example, an acute myocardial infarction is the variation from individual to individual in the number of vulnerable plaques.

The features found in unstable plaques compared with stable plaques have been shown by study of both necropsy and atherectomy material to be a large core of extracellular lipid, a high density of macrophages containing lipid, a reduced smooth muscle content, and a thin cap. The majority of episodes of major thrombi, particularly in white men with high plasma lipids, are due to plaque rupture. In plaque rupture, the fibrous cap of a plaque tears, exposing the highly thrombogenic lipid core to blood in the lumen of the artery. The mechanical strength of the plaque cap is therefore a vital component of plaque stability and depends on the amount and organization of collagen and other connective tissue proteins. Smooth muscle cells exist in lacunae in the plaque cap, where they produce and maintain the connective tissue matrix on which the cap integrity depends. The cap tissue is dynamic, with production of connective tissue matrix proteins by smooth muscle cells being balanced by degradation of the matrix. Both sides of this equation are detrimentally altered by inflammatory processes within the plaque. A reduction in the smooth muscle cell density will inevitably lead to a decline in connective tissue synthesis. There is growing evidence that smooth muscle cell death by apoptosis occurs in plaques, perhaps related to a decline in growth factors needed for their maintenance or to the activity of macrophages in the vicinity producing ROSs. Interferon-γ production by lymphocytes depresses collagen synthesis by smooth muscle cells. Enhancement of the catabolic side of the equation of connective tissue synthesis, however, is probably more important. Connective tissue matrix proteins are degraded by a range of proteases, the most widely studied of which are the metalloproteinase family. There are at least 12 members of this family, with a large range of molecular weights and with considerable individual variation in their affinity for different components of the connective tissue matrix. One form (MT-MMP) is bound to cell membranes, and its activation plays a role in cell migration. Those with the ability to initiate or enhance the degradation of collagen include interstitial collagenase (MMP-1), gelatinase B (MMP-9), and stromelysin (MMP-3). Although several cell lines in the plaque, including smooth muscle cells and basophils, produce metalloproteinases, the major source is the macrophage. A feature common to all these metalloproteinases is that they are secreted into the extracellular milieu as an inactive precursor that is then converted to an active lower-molecular-weight enzyme. The same cell type, although not necessarily the same cell, also produces TIMPS, which bind to and neutralize the active enzyme. Control of the catabolism of connective tissue is potentially exerted at three levels. The first is in the transcription and secretion of metalloproteinases by the macrophage, the second is at the activation point, and the third is at the level of inhibition because of binding of TIMPS to the active enzyme.

Observational studies on human plaque tissue have used either in situ hybridization to show metalloproteinase mRNA or immunohistochemistry to show the metalloproteinase itself. The difficulty of such observational studies is that most of the antibodies used recognize both the active and inactive forms of the metalloproteinase and therefore give no indication of the balance of the active enzyme with its inhibitor. Nevertheless, these observational studies have shown large amounts of, in particular, MMP-9 (gelatinase B) and MMP-3 (stromelysin) in macrophages in unstable plaques. A biological assay of dynamic enzyme activity within the plaque can be made by placing the tissue section on a gelatin sheet and observing where lysis occurs. This approach has confirmed that an excess of active enzyme over its inhibitor is present in unstable human plaques and is maximal at vulnerable areas in the cap.

These data suggest that one potential way of inhibiting or preventing atherosclerotic plaque progression and clinical events is to reduce metalloproteinase production or activation. Although MMP-9 is emerging as a major member of the metalloproteinase family in the context of plaque events, it must be remembered that its proenzyme is constitutively expressed by monocytes and macrophages, for example in fatty streaks, long before any question of instability of the plaque arises. Mecha-
nisms must therefore exist for the upregulation of expression, enhanced release of the proenzyme, or increased extracellular activation. Tumor necrosis factor-α and interleukin-1 are known to upregulate metalloproteinase activity by macrophages in culture and are one way in which enhanced inflammatory activity in the plaque leads to a detrimental effect. The interaction of macrophages with lymphocytes using CD40 and its ligand also upregulates metalloproteinases. Although the classic activation pathway for metalloproteinases in the tissues is by plasmin, there is also now evidence that active metalloproteinases can further activate the proenzymes in the adjacent tissue, that MMP-MT will induce activation, that mast cells may play a role, and finally, that ROSs can lead to direct activation of the proenzyme.

Animal models of atherosclerosis induced by high-lipid diets give a system in which the control of metalloproteinases can be explored more fully. In this issue of Circulation, such a rabbit model is described that suggests new ways in which metalloproteinase expression is induced and enhanced.

Studies of the plaques in situ in the rabbit showed that gelatinase B (MMP-9) was plentiful within the lipid-filled macrophage-derived foam cells. The activity assay carried out by laying the plaque on a sheet of gelatin and looking for lysis showed an excess of the active enzyme over its inhibitors (TIMPS). The model thus far confirmed much of what was already known about MMP-9 in atherosclerosis. The novel aspect is that the plaques themselves and macrophage-derived foam cells taken from the lesions into culture in vitro showed a marked reduction in both the expression of the precursor gelatinase B (MMP-9) and its activated form if the animal had been treated with an ROS scavenger, N-acetyl-l-cysteine. The potential benefit of antioxidant therapy is usually regarded as protecting LDL from the modification usually regarded as protecting LDL from the modification that was used to study the activity of metalloproteinases in plaques after reduction of the plasma lipid levels by reversion to a normal diet. A marked reduction in MMP-9 content and activity with the plaque was also recorded. Taken together, these studies provide a rationale for using a combination of lipid lowering and an antioxidant to inhibit metalloproteinases in human atherosclerosis and thereby reduce the risk of further acute events.

References

Key Words: Editorials - metalloproteinases - free radicals
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Circulation. 1998;97:2382-2383
doi: 10.1161/01.CIR.97.24.2382

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