Atheroma Morphology and Mechanical Strength
Looks Are Important, After All—Lose the Fat

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A cute clinical cardiovascular events remain the main cause of morbidity and mortality in industrialized societies. Corroborating evidence obtained from classical pathological observation and state-of-the-art imaging shows that fatal events are mainly due to thrombosis triggered by exposure of a disrupted atherosclerotic plaque contents, rather than to obliteration of blood flow by the plaque bulk. Thus, the time has finally come to concentrate on the factors that may weaken the structure of a plaque to precipitate its sudden disruption. Recent studies have identified morphological characteristics likely to be associated with a plaque’s tendency to rupture, underlining the possibility to clinically use such hallmarks to predict, control, and monitor plaque evolution.

From a mechanical point of view, the delicate balance of plaque stability is controlled, on one hand, by the intrinsic properties of the tissue and, on the other hand, by the external forces to which the plaque is subjected. On the basis of circumstantial evidence, an increased content of lipid and a decreased content of collagen have been long suspected to decrease the mechanical strength of the plaque; however, no direct proof has been offered so far. In a study published in Circulation Research, Rekhter et al, starting from this hypothesis, manipulated the composition of experimental lesion through dietary lipid loading, and then they examined the first side of this equation, ie, the relationship between collagen and lipid content and the mechanical properties of lesions. By demonstrating such a relation, albeit in an experimental model of plaque rupture and without specifically addressing the mechanisms, Rekhter et al have moved present assumptions regarding plaque stability closer to validation. As an important practical application, this study gives impetus to the development of clinical tests based on morphological characterization of collagen/fat content to predict and monitor the clinical course of individual plaques.

Using the rabbit experimental model of plaque rupture they previously proposed, Rekhter et al show now that hypercholesterolemia determined collagen loss in the lesions. Because this could not be attributed to either defective cross-linking or decreased synthesis, they proposed collagen catabolism as the main mechanism by which lesions lose their collagen. Interestingly, they found that collagen loss was focal, a feature that may have important consequences for formation of a plaque’s weak spots, known as vulnerable to rupture. Collagen loss was also found to occur preferentially in areas rich in macrophage-derived foam cells, in agreement with previous observations that human atheroma tends to rupture in areas with inflammatory infiltrates.

Rekhter’s group proposes that an increased activity of matrix metalloproteinases (MMPs), enzymes found to participate in the physiological and pathological remodeling of vascular matrix, is, at least partially, responsible for degradation of collagen in their plaque rupture model. The activity of macrophage-derived MMPs in the vulnerable shoulders of human atherosclerotic lesions has been previously related to collagen degradation. Although their analyses confirmed an increased MMP expression and activity in the weaker lesions of hypercholesterolemic animals, Rekhter et al deemed the mechanism as unknown. Recent studies offer likely explanations. The macrophage-derived foam cells of hypercholesterolemic rabbit lesions express MMPs in situ and continue to secrete these enzymes when explanted in vitro. Also, we found that intracellular lipid accumulation is associated with a considerable enhancement of matrix-degrading activity, because MMP production by lipid-laden macrophages far exceeds that of their nonlipid-laden counterparts. In vitro incubation with oxidatively modified lipoproteins characteristic for experimental and human atherosclerosis increases MMP expression in both vascular cells and macrophages.

A key feature of the matrix-degrading capacity of macrophage foam cells is that besides secreting MMP zymogens, these cells can further unleash the enzymatic activity of latent MMPs, secreted by themselves or by vascular cells via the concomitant release of reactive oxygen species (ROS). Thus, through the combined release of soluble factors that induce MMP expression in neighboring vascular cells, of their own MMPs, and of activators for MMP zymogens, macrophage-derived foam cells are prime modulators of matrix degradation in the atherosclerotic lesion.

The study of Rekhter et al also suggests, as an additional mechanism for collagen depletion, an apparent loss of smooth muscle cells (SMCs). Although mechanisms were not further pursued, apoptosis was indicated as a possibility. In a recent detailed examination of dietary regulation of cell turnover in rabbit lesions, Kockx et al showed that hypercholesterolemia enhances both cell death and cell replication, and that cholesterol withdrawal reverses these effects. Most replicating cells were macrophages, whereas the macrophage and SMC-rich areas showed comparable apoptosis, with averages

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≈2%, a value significantly lower than the cell loss suggested by the report of Rekhter et al. An increasing percentage of macrophages, through both continued recruitment and as a result of in situ proliferation in hypercholesterolemic conditions, could contribute to an apparent SMC loss. Some of the difference may also be a reflection of the method chosen by Rekhter et al, who estimated cell loss from calculating cell densities. These values would be independently decreased through the known accumulation of extracellular material, lipid and matrix, within hypercholesterolemia-induced lesions. Indeed, the extracellular accumulation of lipid itself is likely related to the mechanical strength of lesions. Although the present report confirmed that the cholesterol ester content of lesions increased, the direct effect of this parameter on the mechanical properties of lesions was not investigated. On the basis of previous in vitro data, presence of cholesterol ester increases the stiffness of lesions. Also, the extent and configuration of the extracellular lipid pool in human atheroma were found to influence the site of rupture.

The study by Rekhter et al emphasizes the relationship between hypercholesterolemia and plaque weakening. However, it is essential to note here that hypercholesterolemia, although known to greatly accelerate formation of experimental lesions, is not necessary for the lipid to accumulate within experimental arterial lesions, and, indeed, there is no direct evidence showing that it is a necessary condition for the development of human atheroma. In his early observations, Lyman Duff reported that lipid accumulation is secondary to subtle morphological changes in response to some kind of injury. Decades later, atherogenesis is still largely regarded as a response of the arterial wall to what may be loosely defined as injury. In the naturally occurring human atherosclerotic lesion, this may be inflicted by hemodynamic, infectious, mechanical, or biochemical insults. Subtle changes in the environment of the vessel wall are sufficient to cause an inflammatory reaction that then fuels atheroma progression.

Lipid lowering has been long deemed a worthwhile treatment for atherosclerosis, and its consequences were investigated in numerous experimental models and clinical trials. One of the better characterized among the plethora of reported beneficial effects is the improvement of endothelial function, found in both animal models and humans, likely through the decrease in redox stress. Improved arterial relaxation may contribute to decrease the number of acute events by subduing suspected mechanical triggers. Direct effects of hypercholesterolemia reversal on the experimental lesion morphology included reduction of the lipid core size, with an accompanying increase in lumen area, increase in connective tissue, especially collagen, and decrease in lipid content in monkeys and rabbits. These changes were interpreted to suggest increased lesion strength. However, this suspected beneficial effect was not tested until the present study, which finally demonstrates the decreased mechanical strength of lipid-laden lesions.

Interestingly, alleviation of the oxidative stress that characterizes atherosclerotic vessels has similar effects. Use of ROS scavengers and antioxidants in rabbit atherosclerosis models, in which a cholesterol-rich diet induces oxidative stress, can duplicate effects of lipid lowering. For example, improvement of endothelium-dependent relaxation can be also obtained with probucol treatment. The favorable effect occurs in spite of sustained elevated plasma cholesterol levels. Probucol treatment in Watanabe rabbits also produces morphological changes similar to those obtained with dietary lipid lowering, ie, decreased lipid and increased cellular and fibrous content of lesions. This particular effect of diminished oxidative stress may be partially due to a decreased matrix degradation by MMPs, because we found that the ROS scavenger N-acetyl cysteine (NAC) decreases expression and activity of MMPs produced by macrophage foam cells of rabbit experimental lesions. The issue of being able to dynamically modify the composition of human atheroma remains difficult to settle. On the basis of the study of autopsy specimens, van der Wal et al proposed that the fibrous and lipid-rich human atherosclerotic lesions are interchangeable, and that the shifts may be determined by the presence of inflammation. Diminishing the plaque’s lipid burden, a major target of oxidative modification, reduces or eliminates inflammation, hence removing the source of redox stress, along with all its many consequences. We are, in fact, proposing that the beneficial effects of lipid lowering occur primarily via reduction of the redox stress in the atherosclerotic arterial wall. We also suggest that interventions that specifically decrease the intraplaque lipid will effectively increase the mechanical strength of plaques.

Evidently, the ultimate purpose of the effort to understand the pathogenesis of acute vascular syndromes is to intervene with new therapies. Although major advances have been made in the last few years, one of which is identification of the fundamental importance of plaque stability, we are still unable to effectively prevent acute syndromes. Among the practical issues that still impede our capacity to provide adequate therapy are the continued limited ability to identify and monitor unstable plaques and the scarcity of experimental models for study of plaque destabilization. However, the research efforts of Rekhter et al have produced a novel experimental model. This has already proved useful for pointing out targets and endorsing efforts for improving vascular imaging. The way a plaque “looks” is, after all, important. There is good reason to hope that soon we will be able to predict the clinical evolution of individual plaques and accurately monitor the effects of therapeutic interventions aimed at strengthening human atheroma.

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