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Elimination of Neoangiogenesis for Plaque Stabilization

Is There a Role for Local Drug Therapy?

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Emerging data suggest that intraplaque hemorrhage is critical in promoting atherosclerotic lesion instability. Because red blood cell membranes are a rich source of free cholesterol and accumulated red blood cells within plaques promote inflammation, intraplaque hemorrhage is associated with expansion of the necrotic core. Plaque hemorrhage results from the development of immature neointimal vasa vasorum. Therefore, it is proposed that molecular therapies designed to eliminate pathologic neovascularization within developing lesions will interrupt the process of hemorrhage and decrease the rate of necrotic core expansion. The elimination of intraplaque neovascularization would involve targeting of pre-existing and new vessel development. The concept of vascular regression has met some success in other neovascular-dependent diseases, including macular degeneration and malignancies. The efficacy of this novel approach is dependent on gaining critical knowledge of the environment required to support development and maturation of the vasa vasorum within varying plaque types. A multitargeted approach involving selective local antiangiogenic agents should contribute to prevention of plaque progression and its clinical consequences. (J Am Coll Cardiol 2007;49:2093–101) © 2007 by the American College of Cardiology Foundation

Approximately 75% of acute coronary events and 60% of recently symptomatic carotid artery disease are caused by disruption of an atheromatous plaque (1–3). Although the underlying mechanism for conversion of an asymptomatic fibroatheroma to a lesion vulnerable to rupture is not established, the significance of intraplaque hemorrhage in lesion stability has recently been proposed as an important contributor (4–6). A series of manuscripts in the current issue of the *Journal* propose the association of hypercholesterolemia with the development of increased vasa vasorum around coronary vessels and intraplaque neovascularization. The newly formed vasculature in the plaque is demonstrated to be incompetent, because red blood cells (RBCs) constantly leak into the microenvironment, exaggerated particularly during intraplaque hemorrhage. The RBC membranes are rich in free cholesterol, and the extent of RBC leakage is suggested to be responsible for the rapid expansion of the necrotic core. It is therefore proposed that elimination of the intraplaque neovascularization should substantially decrease the accumulation of RBC-derived cholesterol in plaques, which may slow the development of the necrotic core and promote plaque stabilization.

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Plaque Volume and Necrotic Core Size Determine the Plaque Vulnerability

Histopathologic features of lesions critical to acute coronary events include plaques with markedly attenuated and inflamed fibrous caps underlined by a relatively large necrotic core. Over 80% of plaques associated with acute rupture demonstrate more than 50% cross-section vascular area narrowing, with greater than 75% narrowing found in at least one-half of lesions (7). Not only are ruptured plaques sizable in circumference, they longitudinally span a median length of 9 mm, resulting in relatively large plaque volumes. Such plaques also contain large necrotic cores, which commonly occupy more than 25% of the plaque area. The necrotic core size and plaque volume interact synergistically, increasing the odds of plaque rupture. This association is so strong that other factors, including inflammation, only marginally contribute to lesion vulnerability. The increased

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Abbreviations and Acronyms

Ang = angiopoietin

RBC = red blood cell

VEGF = vascular endothelial growth factor

plaque burden may not consistently produce obstructive disease, because more often the outward and expansive vascular remodeling obviates any compromise in luminal integrity. The developing necrotic cores accumulate lipids not only from circulating lipoproteins but also from the cholesterol-enriched RBC membranes deposited upon intraplaque hemorrhage (4,6).

Plaque hemorrhage is a fairly common event in coronary atherosclerosis. In patients dying from plaque rupture, intraplaque hemorrhage can be observed in up to 5 other sites of the coronary tree (6). In patients dying from stable severe coronary atherosclerosis, plaque hemorrhages are less frequent. The least number of sites of hemorrhage are seen in patients dying of noncoronary causes or plaque erosion. This suggests that lesions with hemorrhage may be prone to disruption if not adequately treated. These hemorrhagic sites also show accompanying increase in neointimal vasa vasorum.

Intraplaque Hemorrhages Contribute to Enlargement of Necrotic Core

A strong association of the extent of intraplaque hemorrhage (identified by glycophorin A staining, a protein exclusive to the erythrocyte membrane) with increasing necrotic core size has been reported in coronary lesions prone to rupture (4). Whether the relationship of intraplaque hemorrhage and lesion instability offered any clinical significance was further explored in case-control magnetic resonance imaging (MRI) study of 29 patients followed over 18 months. Of these 29 patients, 15 demonstrated MRI evidence of intraplaque hemorrhage in carotid plaques at baseline; the remaining 15 patients with comparably sized plaques did not reveal intraplaque hemorrhage (5). The percent change in plaque volume (6.8% vs. −0.15%; $p = 0.009$) and lipid-rich necrotic core volume (28.4% vs. −5.2%; $p = 0.001$) was significantly higher in the hemorrhage group than in the nonhemorrhage plaques. Further, the patients with intraplaque hemorrhage at baseline were more likely to have new plaque hemorrhage (43% vs. 0%; $p = 0.006$). A follow-up study tested whether plaque characteristics studied by MRI were possible predictors of future ipsilateral cerebrovascular events. In that prospective study design, serial carotid MRI scans were performed every 18 months in 154 asymptomatic patients who had ultrasonically verified 50% to 79% carotid stenosis (8). During a mean follow-up of 3 years, 12 carotid cerebrovascular events occurred, which correlated with MRI characteristics of thin or ruptured fibrous caps (hazard ratio [HR] 17.0; $p \leq 0.001$), intraplaque hemorrhage (HR 5.2; $p = 0.005$), large hemorrhagic areas (HR for every 10 mm^2 increase 2.6; $p = 0.006$), large necrotic cores (HR for every 10% increase 1.6; $p = 0.004$), and large plaque dimensions (HR for every 1-mm increase 1.6; $p = 0.008$). Although not confirmatory, these clinical MRI studies suggested that hemorrhage into the carotid atherosclerotic lesion increases plaque volume and necrotic core size, important hallmarks of plaque vulnerability to rupture.

Evidence of RBC-Derived Cholesterol Accumulation

Experimental studies of simulated hemorrhage in at least the skin (9) and brain (10) suggest complete resolution of the lesion by 7 days to 14 days, depending on the extent of hemorrhage. The increase in erythrophagocytosis by macrophages leads to the eventual development of foam cells (11), which in most tissues outside the coronary vasculature, eventually disappear with no pathologic consequence. One exception, however, involves the maxillary sinus, where increased intrasinus pressure due to drainage obstruction may affect venous and lymphatic drainage outflow and lead to venular microhemorrhages; continued arterial flow into the sinus mucosa further contributes to relatively large localized hemorrhages (12). In that circumstance, the lymphatic drainage may be insufficient to completely remove the lipid components of the RBC, and cholesterol crystals precipitate. Hemosiderin-laden macrophages and occasional multinucleated foreign-body giant cells surround the cholesterol clefts in maxillary sinus.

In contrast to organs, the removal of the lipid components of RBC membranes in atherosclerotic plaques may parallel that of the maxillary sinus, because phagocytic clearance of dead macrophages in advanced lesions is inherently defective (13–15). It is presumed that the rapid influx of macrophages in response to the hemorrhage itself would lead to postapoptotic macrophage necrosis and enhanced inflammation in atherosclerotic lesions (16). The cumulative effect of a late lesional hemorrhagic event leads to the generous expansion of the necrotic core, which together with the proinflammatory response of surviving macrophages promotes further inflammation, plaque instability, and thrombosis.

As proof of principle, we developed an animal model simulating intraplaque hemorrhage to assess the role of RBC-derived cholesterol in lesion progression (4). The direct injection of 25 μl to 50 μl packed RBCs into quiescent aortic atherosclerotic plaques produced excessive macrophage infiltration along with free cholesterol crystals and hemosiderin-laden macrophages. In contrast, control (noninjected) lesions showed the characteristics of a regressed lesion with far fewer lesional macrophages and no free cholesterol. Neutral lipids identified by oil red O were also significantly greater in injected plaques.

The pathologic response to the extravasation of blood involves a cellular reaction in tissues adjacent to where monocyte/macrophages are strongly drawn to the lesion. The signals for this perifocal migration of inflammatory cells are not fully understood, but proteins in the coagulated blood likely contribute to cellular activation (17,18). Alter-