

EDITORIAL COMMENT

Coronary Plaque Neovascularization and Hemorrhage

A Potential Target for Plaque Stabilization?*

Aloke V. Finn, MD,† Rakesh K. Jain, PhD‡

Atlanta, Georgia; and Boston, Massachusetts

Angiogenesis, or the growth of new blood vessels from existing host vessels, is increasingly being recognized as important in the growth and progression of atherosclerosis, the primary cause of coronary artery and cerebrovascular disease. Neovascularization of atherosclerotic plaques was first noted by Koester (1) in 1876. Later Barger et al. (2) proposed that the growth and extension of adventitial blood vessels called vasa vasorum (VV) into the intima occurs as a response to

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tissue hypoxia, which occurs when the intima thickens beyond the diffusion limits of oxygen and nutrients (approximately 350 μ M) (3). Indeed, increases in hypoxia-inducible factor alpha, a transcription factor that is up-regulated under hypoxic conditions and promotes hypoxia-dependent neovascularization, have been found in human atherosclerotic plaques (4).

Because many of the VV that grow into the plaque are immature (i.e., they lack mural cells and competent endothelial cell [EC] junctions), they are inherently leaky, permitting inflammatory cell infiltration and influx of blood constituents, especially erythrocytes, into the plaque. Most of the intraplaque vessels are endothelialized, but only a few possess mural pericytes and smooth muscle cells (5,6). The lack of

mural cells and poorly formed EC junctions contribute to the incompetence of immature intraplaque VV. Erythrocyte membranes, rich in phospholipids and free cholesterol, and free hemoglobin, a source of oxidative damage and reactive oxygen species, contribute significantly to necrotic core expansion and inflammatory cell infiltration, 2 features essential to all high-risk plaques (7).

Neovessels also promote the entry of leukocytes into the plaque by up-regulation of adhesion molecules, such as intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin (8). Increased matrix metalloproteinase release from activated macrophages and proteases secreted from mast cells cause further damage to microvessels and facilitate intraplaque hemorrhage (9).

In this issue of *JACC*, work by Gössl et al. (10) lends further support to the concept that coronary VV neovascularization plays an important role in plaque progression through promotion of intraplaque hemorrhage. Using hearts from 15 patients obtained by autopsy, they demonstrated that VV density was higher in nonstenotic (defined as <50% lumen diameter stenosis) and noncalcified plaques compared with normal segments. As expected, the amount of iron and glycophorin A (an anion-exchange protein specific to erythrocytes) was significantly higher in nonstenotic and stenotic plaques compared with normal segments and correlated with VV density.

Perhaps more novel and interesting is their finding that in calcified stenotic plaques, VV spatial density was lowest; however, relatively high amounts of glycophorin A and iron were found in these lesions, suggesting that intraplaque hemorrhage is associated with plaque calcification.

These findings confirm earlier work linking hemorrhage to coronary plaque progression. In a relatively large number of human coronary plaques from victims

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From the †Department of Internal Medicine, Emory University School of Medicine, Atlanta, Georgia; and the ‡Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts. Dr. Jain was supported in part by NIH grants R01-CA85140, R01-CA115767, R01-CA126642, and P01-CA80124. Dr. Jain has received consulting fees from Dyax, AstraZeneca, Millennium, and Enlight Biosciences; lecture fees from Pfizer and Roche; grant support from Dyax and AstraZeneca; and owns equity in SynDevRx and Enlight Biosciences.

of sudden coronary death, Kolodgie et al. (11) demonstrated that there was a greater frequency of previous intraplaque hemorrhages in coronary atherosclerotic lesions prone to rupture compared with early lesion morphologies or stable plaques. Importantly, the extent of intraplaque hemorrhage corresponded to the size of the necrotic core, suggesting that deposition of red cell membranes—rich in free cholesterol and phospholipids—is an important cause of core expansion and plaque progression. Although additional information about the type of plaque morphology included in the nonstenotic plaque group would have strengthened the study of Gössl et al. (10) by enhancing our understanding of how neovascularization and hemorrhage contribute to plaque progression, quantitation of VV density using micro-computed tomography (CT) images represents a significantly novel aspect of this study. Prior quantitation of VV density histologically has also been shown to correlate with plaque progression and reinforces the authors' conclusions (12).

The association of intraplaque hemorrhage with plaque calcification is a more novel aspect of this study. Although coronary artery calcification correlates with the severity and extent of coronary disease at autopsy (13), there does not appear to be a linear relationship between coronary calcium and risk for future coronary events (14). Pathologically, ruptured plaques do demonstrate significantly higher calcium levels than do thin cap fibroatheromas (i.e., vulnerable plaques), but the causes for this remain unknown (15). This same relationship holds true for mean number of VV and for hemosiderin-laden macrophages (another sign of previous hemorrhage) (12); therefore, it is tempting to speculate that increases in coronary calcium levels seen in ruptured plaque may result from hemorrhage. However, neither study demonstrates a cause and effect phenomenon, which is essential to furthering our understanding of the relationship of coronary calcification and plaque stabilization. Instead, it begs the question of how and why heavy calcification occurs in human coronary atherosclerotic disease and whether it is simply a healing response to hemorrhage.

Although not specifically addressed in this study, another important and related question involves understanding how and why hemorrhage occurs in human coronary atherosclerotic lesions. Recently, Sluimer et al. (6) demonstrated abnormalities such as incomplete endothelial junctions and basement membrane detachment in human intraplaque ECs. In addition, there was monocyte and mast cell accumulation in these areas, substantiating the con-

cept that intraplaque microvessels are abnormal and thus provide entry points for erythrocyte and other blood cell components into the plaque. However, no change in mural cell coverage was observed from early, advanced, and ruptured coronary plaques, suggesting lack of mural cell coverage alone may not be responsible for intraplaque hemorrhage. This paradigm, that pathologic angiogenesis within plaques leads to hemorrhage, would be strengthened by *in vivo* imaging data.

Further work needs to be done to understand precisely how and which molecules regulate the key pro-angiogenic and antiangiogenic factors in the VV within the plaques that show hemorrhage. The mechanisms responsible for the abnormal EC morphology seen in intraplaque vessels undoubtedly involve vascular endothelial growth factor (VEGF). Angiogenesis depends on EC invasion and proliferation as well as pericyte coverage of vascular sprouts, processes that are coordinated by VEGF and platelet-derived growth factor (PDGF) (16). VEGF disrupts EC junctions and disrupts vascular smooth muscle cell function by inhibiting PDGF-induced pericyte coverage of nascent vascular sprouts (17,18). Placental-like growth factor (PlGF) also stimulates vascular permeability, and its expression has been associated with plaque vulnerability (19). The Tie receptors, Tie1 and Tie2, and 2 ligands for Tie2, Ang1 and Ang2, are also critical for vessel formation and maturation. Sources of Ang1 and Ang2 are mural cells and ECs, respectively. Ang1 is known to stabilize nascent vessels and make them leak resistant, presumably by facilitating communication between ECs and mural cells. However, the mechanism of vessel maturation by Ang1 is far from clear. The role of Ang2 appears to be contextual. In the absence of VEGF, Ang2 acts as an antagonist of Ang1 and destabilizes vessels, ultimately leading to vessel regression. In the presence of VEGF, Ang2 facilitates vascular sprouting. A high Ang2/Ang1 ratio has been found in vulnerable neovascularized plaques (20). Other molecules and growth factors have also been shown to play a role in vessel maturation.

Another avenue of interest raised by the present study is whether or not it might be possible to prevent, destroy, or "normalize" neovessels within plaques. This topic has many parallels in other fields of medicine, especially oncology. Given the dependence of tumor growth and metastasis on blood vessels, inhibition of new vessel formation or destruction of existing vessels of tumors has been one of the most exciting areas of cancer research for the past decade. Most of the clinical trials to date have tested agents that neutralize VEGF or inhibit its signaling.

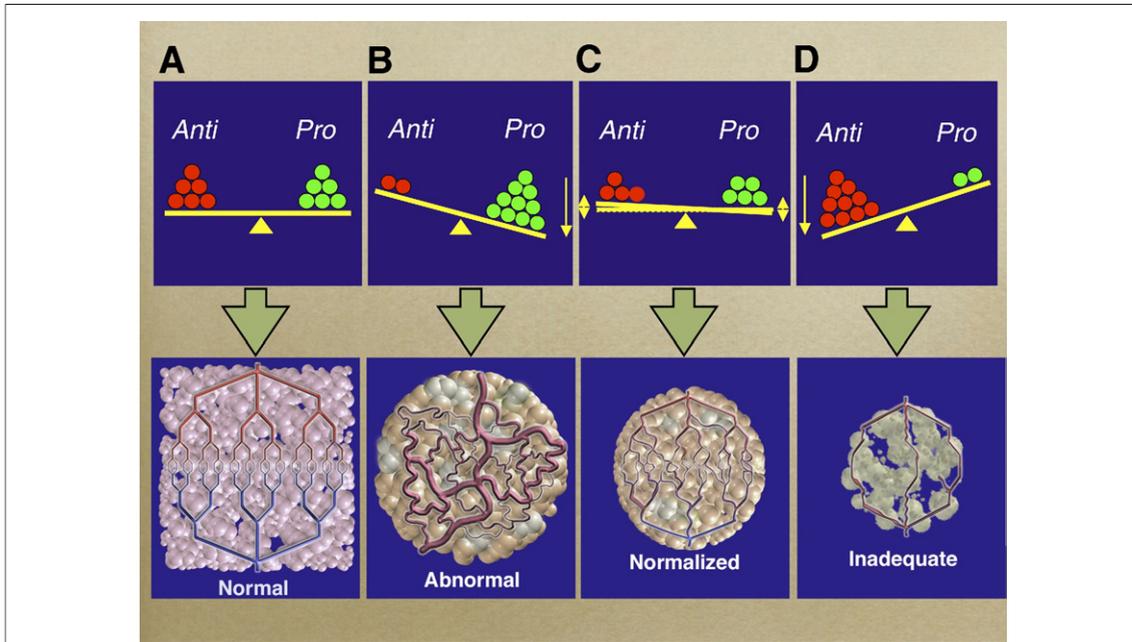


Figure 1. Normalization Hypothesis

(A, top and bottom) In normal tissue, the effect of pro-angiogenic molecules (e.g., vascular endothelial growth factor, basic fibroblast growth factor, interleukin-8) is balanced by that of antiangiogenic molecules (e.g., thrombospondin-1 and -2). Therefore, blood vessels in normal tissues are abnormal structurally and functionally, often leading to hypoxia. More than 70 diseases are characterized by abnormal vasculature (e.g., cancer, atherosclerosis, macular degeneration). (C) Our hypothesis is that judicious administration of effective antiangiogenic agents can “normalize” abnormal vessels and make them less leaky, resembling normal vasculature. (D) Higher doses of potent antiangiogenic agents may be able to completely destroy these abnormal vessels, but they may also harm the vasculature of some normal tissues. This “Janus” effect is one reason we propose to use lower doses of antiangiogenic agents rather than higher doses that can completely inhibit angiogenesis. Reprinted with permission from the AAAS (Jain et al. [21]).

We have recently proposed—and demonstrated both pre-clinically and clinically—that antiangiogenic agents can prune and “normalize” the abnormal vasculature of tumors (Fig. 1) (21). Based on similarities to the structure and function of tumor vessels, could the judicious application of antiangiogenic agents prune and normalize immature intraplaque VV, thereby preventing intraplaque hemorrhage (22)?

The successes of anti-VEGF strategies in cancer biology raise the intriguing question of whether such therapy may be applicable to atherosclerosis. However, this strategy may hold more potential pitfalls than promises given the increased risks of atherothrombotic events seen with systemic antiangiogenic therapies and the reliance of coronary collateral formation on similar angiogenic pathways. Newer agents that might normalize abnormal vasculature without affecting healthy vessels such as anti-PlGF antibodies

are currently being developed and may be a more tenable strategy (23,24).

In conclusion, the work of Gössl et al. (10) lends further insights into the association of neovascularization and hemorrhage with coronary atherosclerosis. Further work needs to be done to understand the causal relationship between hemorrhage and plaque calcification. Until the regulatory molecular mechanisms of intraplaque microvessel formation are better understood, we cannot be sure that the 2 are related or just coincidental bystanders. These and other recent data suggest that strategies to “normalize” intraplaque neovessels to prevent hemorrhage may be promising new avenues for the prevention of coronary events.

Reprint requests and correspondence: Dr. Alope V. Finn, Department of Internal Medicine, Emory University School of Medicine, Emory Crawford Long Hospital, 550 Peachtree Street NE, Atlanta, Georgia 30308. E-mail: avfynn@emory.edu.

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