Plaque Neovascularization and Antiangiogenic Therapy for Atherosclerosis

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The concept that neovascularization of the vessel wall may play a fundamental role in the pathophysiology of atherosclerosis was proposed more than a century ago. In recent years, supportive experimental evidence for this hypothesis (such as the finding that neointimal microvessels may increase delivery of cellular and soluble lesion components to the vessel wall) has been underscored by clinical studies associating plaque angiogenesis with more rapidly progressive high-grade disease. Attention has also focused on a possible role for microvessel-derived intraplaque hemorrhage in the development of acute lesion instability. The interest of clinicians in this phenomenon has been spurred by the potential to target vessel wall neovascularization with angiogenesis inhibitors, a therapeutic approach that has been associated with impressive reductions in plaque progression in animal models of vascular disease. The rationale for pursuing an “antiangiogenic” strategy in the treatment of patients with vascular disease, and a framework for further preclinical evaluation of such therapy, is presented here. (J Am Coll Cardiol 2007;49:2073–80) © 2007 by the American College of Cardiology Foundation

Accumulating evidence has linked plaque angiogenesis with progressive atherosclerotic disease and the development of acute lesion instability. However, no consensus has yet emerged as to whether a true causal association exists. The implications of this debate for clinical practice are significant. Burgeoning interest in proangiogenic gene and cell therapies is tempered by concerns regarding the potential for these therapies to accelerate and/or destabilize atherosclerotic disease. In contrast, the availability of a growing number of “antiangiogenic” agents holds the potential for a new therapeutic paradigm in vascular disease, targeting plaque microvasculature with the aim of slowing plaque growth and promoting plaque stability. This approach has already shown promising results in cancer therapy and in other conditions such as macular degeneration and rheumatoid arthritis, where a role for angiogenesis in disease progression is increasingly recognized. As our ability to modulate angiogenesis in vivo expands, these therapeutic opportunities and potential pitfalls merit careful consideration in the context of the overall debate surrounding vessel wall neovascularization and atherosclerosis.

In this review we examine the pathophysiologic and experimental basis for therapeutic targeting of the plaque microvasculature and present a framework for future preclinical investigation as this treatment strategy evolves. Throughout, the terms “antiangiogenic” and “proangiogenic” (or similar) are used in relation to agents that are considered to have demonstrated such activity in vitro and/or in vivo. However, it should be acknowledged at the outset that our understanding of the regulators of neovascularization in vivo remains incomplete and, indeed, that a number of putative molecules have failed to perform as expected in clinical trials (1,2).

Neovascularization of Atherosclerotic Arteries
Vasa vasorum-derived microvessels do not extend to the intima of normal arteries, penetrating only the adventitia and outer media (3). Diffusion of oxygen and other nutrients is limited to 100 μm from the lumen of the blood vessel, which in normal arteries is adequate to nourish the inner media and intimal layers. As vessel wall thickness increases in the setting of vascular disease, proliferation of the vasa vasorum and intimal neovascularization is observed.

Accumulating histopathologic data associates plaque angiogenesis with more rapidly progressive and unstable vascular disease (4). Briefly, these studies confirm intimal neovascularization to be an almost ubiquitous feature of atherosclerotic disease, correlating with both histologic grade (5,6) and symptoms (7). The majority of microvessels arise from the adventitial vasa vasorum (8,9) and rarely from the luminal surface of the parent artery (8). Microvessel density is greatest in lesions with marked macrophage

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infiltration of the fibrous cap (10), in lipid-rich lesions (6,11), and in thin-cap atheroma (10), all features of so-called vulnerable plaque (12). Rupture is most frequently identified at the shoulder of such lesions, and increased density of plaque microvessels has been identified at this site (10,13). Finally, the presence of angiogenesis at the base of the plaque has been independently correlated with plaque rupture, underscoring the potential for a direct contributory role of neovascularization in this process (10).

**Mechanisms of Vessel Wall Neovascularization**

The process of new blood vessel growth is frequently referred to as angiogenesis. More recently, use of this term has been narrowed to define a process characterized by extension of an existing vascular bed by sprouting of new capillaries from postcapillary venules. Therefore, in this paper, the term “angiogenesis” does not encompass the entire spectrum of events that can result in new blood vessel development, such as arteriogenesis (large-caliber collateral vessel formation) or vasculogenesis (de novo formation of new blood vessels by in situ differentiation of primitive stem cells). Initial steps in angiogenesis include an increase in vascular permeability and proteolytic degradation of the surrounding extracellular matrix. This is followed by chemotactic migration and proliferation of endothelial cells, formation of a lumen, and functional maturation of the newly formed capillary by tightening of interendothelial cell junctions.

The molecular mechanisms underlying vessel wall angiogenesis in vascular disease are now being elucidated (Fig. 1). Hypoxia is one of the most potent stimuli for angiogenesis, and zones of hypoxia are present within thickened atherosclerotic plaque (14). Hypoxia-induced neoangiogenesis is regulated by hypoxia inducible factor-1 (HIF-1), a heterodimeric transcription factor composed of a HIF-1-alpha and HIF-1-beta subunit. In order to respond rapidly to hypoxia, cells continuously synthesize, ubiquitinate, and degrade HIF-1-alpha protein under normoxic conditions. Under hypoxic conditions, such degradation is inhibited, resulting in accumulation of the protein, dimerization with HIF-1-beta, binding to hypoxia-responsive elements within target genes, and activation of transcription via recruitment of the coactivators p300 and CBP (15). The expression of more than 40 genes is known to be activated at the transcriptional level by HIF-1, including those responsible for production of nitric oxide synthase and vascular endothelial growth factor (VEGF) (15). The initial step in angiogenesis involves nitric-oxide-mediated vasodilation. Vascular permeability subsequently increases in response to VEGF, allowing extravasation of plasma proteins that lay down a provisional scaffold for migrating endothelial cells (1). Thus, it appears likely that hypoxia within the plaque core is an important trigger of proangiogenic activity in established vascular lesions that exhibit significant neointimal thickening.

A role for vessel wall hypoxia and plaque angiogenesis in early atherosclerotic disease has been studied, but not yet firmly established. Up-regulation of proangiogenic growth factors (including VEGF) within the vessel wall may precede intimal thickening (4), and, indeed, coronary neovascularization in hypercholesterolemic swine precedes the development of endothelial dysfunction (16). Furthermore, experimental occlusion of adventitial vasorum may lead to intimal lesions that resemble atherosclerosis (17). Together, these hypothesis-generating data suggest that impaired perfusion of the vessel wall (owing to adventitial microvascular dysfunction) may be an alternative mechanism leading to vessel wall hypoxia, even in the absence of neointimal thickening, and may be relevant in the initiation of plaque development. Further pathophysiologic insights in this area have the potential to generate valuable new concepts of early vascular disease, with possibilities for therapeutic development.

Adding to the complexity of this biology, hypoxia-independent pathways for modulation of angiogenesis within the vessel wall have also been identified. For example, hypertension may induce vessel wall neovascularization by hypoxic activation of the HIF-1 system (owing to medial thickening) (18), but direct induction of VEGF expression by smooth muscle cells exposed to stretch has also been demonstrated (19). Oxidative stress and nicotine may modulate growth factor gene expression in vascular cells independent of the presence of hypoxia (20–24).

The relative importance of hypoxia-dependent and -independent pathways in proangiogenic growth factor gene expression in vascular disease has yet to be defined. However, evidence that downstream mediators of angiogenesis are active in the diseased vessel wall is not in doubt; growth factors such as VEGF, hepatocyte growth factor, and platelet-derived growth factor are abundantly expressed within atherosclerotic lesions (5,25–30). Recognized sources of these growth factors include intraplaque cells (such as phenotypically modified smooth muscle cells and inflammatory cells) and the extracellular matrix (31–36) (Fig. 1). Importantly, the distribution of such growth factors has been correlated spatially and quantitatively with vessel wall neovascularization (5,25). Moreover, early administration of VEGF or fibroblast growth factor in experimental models of vascular disease has been associated with accelerated neovascularization of the vessel wall and more aggressive lesion development (37–39). Collectively, these data support a role for proangiogenic growth factor expression in plaque progression.

Increased expression of proangiogenic growth factors may not, however, be sufficient to induce angiogenesis in isolation. This is because of the countervailing influence of endogenous inhibitors of angiogenesis such as the throm-
bosponsins (40), platelet factor-4 (41), endostatin (42), and kallistatin (43) (Fig. 1). The competitive balance between these agonists and inhibitors has been the subject of intense study in tumor biology. Less is known at present about their role in vascular disease, although experimental animal models support a role for endogenous systems of angiogenesis inhibition as a regulatory mechanism in plaque growth. For example, deficiency of collagen XVIII and its proteolytically released endostatin fragment (normally found in vascular basement membranes and the walls of major blood vessels) is associated with enhanced intimal neovascularization and more aggressive atheroma formation (44). Furthermore, single-nucleotide polymorphisms of thrombospondin-1, -2, and -4, which are associated with reduced plasma levels of thrombospondin, are also associated with increased risk of premature myocardial infarction (45). Most notably, evidence that endogenous inhibitors of angiogenesis can be down-regulated in vivo is now emerging (46). Notwithstanding these intriguing findings, there is as yet no conclusive evidence from human studies to support dysregulation of an “angiogenic switch” within the vessel wall characterized by increased proangiogenic growth factor activity and accompanied by down-regulation of endogenous inhibitors.

Figure 1 Mechanisms of Vessel Wall Neovascularization in Atherosclerosis

Increased proangiogenic activity, which may be accompanied by reduced endogenous inhibition of angiogenesis, results in vasa vasorum proliferation and intimal neovascularization. FGF = fibroblast growth factors; HGF = hepatocyte growth factor; PDGF = platelet-derived growth factor; SMC = smooth muscle cell; VEGF = vascular endothelial growth factor. Copyrighted and used with permission of Mayo Foundation for Medical Education and Research.
Putative Roles of Plaque Angiogenesis in Vascular Disease

Conduits for cellular and soluble lesion components. A fundamental paradigm of vascular disease has for many years presumed the artery luminal endothelium to be the key portal for cellular and soluble lesion components (such as lipid, cytokines, and growth factors) found in atherosclerotic plaque (47). However, it is plausible that intraplaque vessels may also function as an important endothelial interface in the recruitment of these lesion components. This concept is more understandable when one considers the significantly greater endothelial surface area and lower blood flow velocities through these microvessels when compared to that of the macroarterial system, albeit that the absolute level of blood flow through this microvasculature is still uncertain.

Studies by Heistad et al. (48) have raised an interesting physiologic dilemma in this regard, whereby intraplaque hydrostatic pressure estimated around 100 mm Hg did not appear to significantly compromise flow through this circuit from adventitial arteries, which are likely to exhibit a mean intravascular pressure of no more than 40 mm Hg. Nevertheless, increased solute delivery to the vessel wall by vasa vasorum in hypercholesterolemia has been demonstrated (49), lending further support to a conceptual framework whereby plaque microvessels might transport lipid as well as cells to the vessel wall. Metabolic substrates required for ongoing smooth muscle and inflammatory cell activity might also be provided, permitting growth beyond the threshold whereby diffusion from the artery lumen is insufficient to meet the metabolic demands of the plaque (Fig. 2).
Developing animal models to definitively prove such a role for microvessels within the artery wall has been extremely challenging, particularly with regard to a role in cell delivery to the plaque. The spatial and quantitative correlation of intraplaque vessels with focal collections of inflammatory cells could occur because these cells stimulate angiogenesis and/or because the plaque microvasculature is particularly active in the delivery and recruitment of inflammatory cells (50) (Fig. 2). In support of the latter interpretation is the finding that expression of leukocyte adhesion molecules is more prevalent on intimal microvascular endothelium than on arterial luminal endothelium and that the presence of these adhesion molecules on neovessels is strongly associated with increased leukocyte accumulation (11,51). Moreover, use of angiotatin in a murine model of atherosclerosis was found to reduce plaque microvessel density and inhibit atherosclerosis development (50) and lead to reduced plaque macrophage content (50).

The latter study has been criticized on the basis that mouse aortas are relatively small with a thin media and may not accurately model human vascular disease, where the limit of diffusion (approximately 100 μm) is likely to be of greater functional relevance (52). Only a minority of lesions in this study exhibited neovascularization (50), in keeping with a lesser role for vessel wall neovascularization in murine atherosclerosis. Other beneficial effects of angiostatin may have accounted for the impressive reductions in disease progression that were observed, but they should not detract from the overall findings of this study. Validation of this therapeutic approach in large animal models will be of immense interest as the field moves forward.

**Intraplaque Hemorrhage**

Intraplaque hemorrhage is increasingly recognized as a key mechanism through which atherosclerotic plaque may expand rapidly and/or rupture (53) (Fig. 3). Plaque neovessels are thin walled, usually without investment by smooth muscle cells or pericytes, and are often of quite large caliber when compared to normal capillaries (3). This fragile structure could reasonably be regarded as sufficient in itself to render these vessels prone to hemorrhage, and indeed, the presence of intraplaque hematoma has been colocalized with these vessels and not intimal disruption, implicating them (and not the artery lumen) as the source of such hemorrhage (53). The added presence of matrix-degrading enzymes produced by macrophages and mast cells, which are frequently found in close proximity to these fragile microvessels, may further exacerbate their tendency to rupture (53). Experimental study of the role of intraplaque hemorrhage in plaque rupture has in the past been hampered by a lack of suitable animal models, although a recently described murine model of plaque rupture may provide new opportunities in this regard (54).

**Other Proatherogenic Mechanisms**

Expression of matrix metalloproteinases is increased in vulnerable regions of human atherosclerotic plaque (55) and is a mechanism that may contribute to the development of plaque rupture. The finding of high neovascularization densities in these same unstable plaque regions suggests that angiogenesis may be an important source of this matrix-degrading activity (56). Increased vasoreactivity has also
been observed in diseased coronary segments and is a factor that has been implicated in the genesis of acute coronary syndromes. It has been suggested (49) that increased vessel wall blood supply related to plaque angiogenesis in these segments may influence the predilection to spasm by providing higher local concentrations of circulating catecholamines and other vasoactive agents (57). The role of coronary vascular tone in the etiology of microvessel rupture is unknown but may be another mechanism through which coronary spasm may contribute to the development of acute coronary syndromes.

**Antiangiogenic Therapy for Atherosclerosis**

Considerable interest is focusing on a treatment approach targeting inhibition of microvessel formation and/or function within atherosclerotic plaque. More than 300 potential inhibitors of angiogenesis have been identified, of which 80 are currently being tested in clinical trials (58). Their mechanisms of action are varied, affecting aspects of angiogenesis such as endothelial cell proliferation, the availability or production of endothelial cell growth factors, the signaling of tyrosine kinase receptors on endothelial cells, and the activity of metalloprotease enzymes. Although significant differences in efficacy between agents may not be apparent in a heterogeneous patient group, it is possible that subpopulations such as diabetics may ultimately benefit from tailored therapy that takes account of specific signaling or other molecular defects of angiogenesis known to be more prevalent in these patients (59). Combination therapy using 2 or more inhibitors with differing mechanisms of action may also prove necessary to achieve maximal therapeutic effect.

The first antiangiogenesis clinical trials in cancer patients did not live up to their high expectations, in part because the advanced tumors being treated had already activated various pathways that allowed them to easily override the angiogenic restrictions of a single inhibitor (60). It would be prudent to anticipate similar obstacles in the antiangiogenic treatment of atherosclerosis.

That angiogenesis inhibitors may favorably influence plaque microvessel function by inducing functional maturation of these vessels is another intriguing possibility (61). Reduced vessel “leakiness” in response to antiangiogenic therapy has been demonstrated in cancer studies, enhancing chemotherapeutic drug delivery to the tumor and decreasing the risk of metastasis (62). Similar effects on plaque microvascular integrity could play an important role in reducing risk of intraplaque hemorrhage. Microvascular “maturation” might also facilitate the development of therapies targeting plaque regression. For instance, improved microvascular exchange might facilitate high-density lipoprotein cholesterol-mediated “reverse cholesterol transport” from lesions or the trafficking of monocyte-derived cells from atherosclerotic plaque. The latter has recently been documented to occur during lesion regression (63). Improved delivery of novel agents that influence plaque biology (targeting the inflammatory milieu or the function of phenotypically modified vascular smooth muscle cells) may also be a favorable consequence of microvascular maturation, analogous to the synergy that has been observed between antiangiogenic and cytotoxic therapies in cancer (64).

An ability to optimize the choice and dose of angiogenesis inhibitor using reliable surrogate markers of efficacy may be critical to success in the clinic. Optimal dosing of assorted protein fragment statins, for example, is likely to be challenging, given that such fragments may already be variably expressed within the proteolytic environment of the plaque. Novel circulating markers of angiogenesis show promise (65), as do increasingly sophisticated imaging techniques (such as molecular imaging using magnetic resonance imaging) that may directly quantitate vessel wall neovascularization in vivo (66,67). Detection of changes in artery wall oxygenation may be possible using positron emission tomography with 18-fluoromisonidazole or magnetic resonance imaging (64). High-resolution imaging can also identify complications of plaque angiogenesis such as intraplaque hemorrhage (68), a potentially valuable index of therapeutic efficacy. Ultimately, such tools may not only assist in selection and titration of antiangiogenic therapy, but might also identify patients most likely to benefit in the first instance.

The greatest concern regarding use of angiogenesis inhibitors in atherosclerosis is undoubtedly the potential for these agents to aggravate preexisting end-organ ischemia. Timing of therapy may be crucial, with no adverse effect of angiogenesis inhibitors on collateral development when treatment was delayed for some time after vascular occlusion (69). Other concerns include the possibility that microvessel “maturation” may in fact promote disease progression by improving delivery of oxygen and nutrients to the vessel wall (64). Interestingly, experimental and clinical studies in tumor biology do not indicate that lesion growth accelerates during antiangiogenic monotherapy. The reasons for this are poorly understood but may suggest a predominance of microvessel regression over microvessel maturation as the mechanism of therapeutic benefit (64).

On a final cautionary note, it should be recognized that our understanding of these complex agents and their effects in vivo, and in a variety of disease settings, is still at a very early stage. Recent clinical experience with cyclooxygenase-2 inhibitors, in particular, has emphasized that although some angiogenesis inhibitors may prove to have a protective function in the cardiovascular system, these findings may not pertain to all agents with angiogenesis activity (70). Already, certain agents have been associated with the development of a prothrombotic state (71) and hypertension (72). Direct cardiotoxicity leading to severe congestive heart failure has recently been described as a rare complication of imatinib therapy (73). Together, these data underscore an absolute requirement for rigorous experimental evaluation of this treatment paradigm before we can begin to consider the possibility of clinical trials.
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