Thrombin and Other Potential Mechanisms Underlying Restenosis

Josiah N. Wilcox, PhD

Percutaneous transluminal coronary angioplasty (PTCA) has become important in the management of coronary artery disease; however, proliferative restenosis in 25–35% of the patients undergoing PTCA limits its usefulness. The pathogenesis of the postangioplasty restenosis lesion has not been defined. Morphologically, the lesion largely comprises smooth muscle cells (SMCs) and develops in the first 3 to 6 months after balloon angioplasty. Little is known about the time course of cell proliferation in the human lesion, including where cell proliferation begins or why proliferation stops in some people but continues in others. Some degree of smooth muscle proliferation is a likely part of the healing process after injury; however, it is unclear why some individuals develop clinically significant lesions. Morphological observations, assembled from human vessels after balloon catheterization, have been primarily descriptive with few insights into the causes or the time course of the development of the postangioplasty restenosis lesion. However, one common finding of these studies has been the presence of intimal or medial tears with concomitant thrombus formation on the injured surface.1-4

See p 232

Advanced human atherosclerosis is characterized by intimal SMC proliferation and the accumulation of lipids and inflammatory cells, including macrophages and T cells.5,6 Commonly, the critical event converting asymptomatic atherosclerotic plaques into symptomatic lesions is thrombosis,7-9 whereas nondiseased, uninjured arteries are not thrombogenic. It has been suggested that plaque rupture is the integral event that precipitates thrombus formation.10-13 Plaque rupture or cracking is often found associated with thrombus formation in both the coronary7,14,15 and cerebral arteries,16 and these thrombi often extend into the region of the necrotic core of the plaque through such cracks. Tissue factor (TF) is synthesized in atherosclerotic plaques and significant deposits are found in association with the necrotic core and fibrous cap,17 regions subject to exposure by plaque rupture. TF may therefore initiate the formation of thrombus upon rupture of human atherosclerotic plaques. Plaque rupture with exposure of TF-rich intima is produced by PTCA so thrombus formation on the vessel surface is a natural consequence of PTCA. For this reason, patients undergoing PTCA are often administered antithrombotics after surgery. However, despite the administration of heparin and aspirin after PTCA, both early thrombotic occlusions and later occlusions due to development of postangioplasty restenosis lesions continue to complicate this procedure.

The article by Sarembock et al18 in this issue of Circulation provides new clues regarding the development of the postangioplasty restenosis lesion. Lipid-rich atherosclerotic plaques in rabbits were subjected to balloon catheter angioplasty (BCA), and the animals were subsequently treated for 2 hours with either heparin or recombinant hirudin, a specific and potent inhibitor of thrombin. Both groups showed an early improvement in luminal diameter after BCA; however, 28 days after BCA, the hirudin group had a significantly larger lumen and less intimal lesion development compared with the animals receiving heparin alone. These results are very exciting in that they suggest that 2 hours of antithrombin treatment may modify lesion development after vascular injury. However, the mechanism by which hirudin reduced lesion development remains to be determined.

Roles for fibrin deposition and thrombus organization in the development of human atherosclerotic plaques have recently been reemphasized.19 The “thrombogenic” or “encrustation” hypothesis originally proposed by von Rokitansky in the 1800s suggested that plaques might develop through the abnormal deposition of fibrin or blood products on the surface of blood vessels. Additional support for this hypothesis came with the observation by Duguid20 of a continuum between organizing thrombi and fibrotic intimal thickening in many atherosclerotic plaques. The concept that fibrin clots in plaques may contribute to cell proliferation through some effect on surrounding smooth muscle cells is supported by studies examining organization of chronic
arterial thrombi in rats, rabbits, and pigs. In all of these studies, arterial thrombi in contact with the vessel wall eventually developed into fibrous intimal thickenings as part of the normal healing process.

Platelet-derived growth factor (PDGF) is an important growth factor that may stimulate intimal smooth muscle proliferation in atherosclerotic plaques in humans. Since PDGF is a major component of the platelet and is released with platelet binding and activation, it was suggested that platelet release at sites of vascular injury and thrombosis might contribute to intimal smooth muscle proliferation. Local synthesis of PDGF may also be important to continued intimal proliferation. Both PDGF and PDGF receptors are synthesized locally in atherosclerotic plaques by smooth muscle cells and a population of mesenchymal-appearing intimal cells (MICs), and these cells are often found in human atheroma associated with regions of focal organizing thrombi.

Cyclin (proliferating cell nuclear antigen), a DNA polymerase auxiliary protein, is present during G1, S, and G2 phases of the cell cycle and cyclin immunohistochemistry has recently been used to label proliferating cells in human atherosclerotic plaques. Many plaques showed a very low (less than 1%) replication rate, whereas some had labeling indexes greater than 4%. The proliferating cells in these tissues were smooth muscle cells and macrophages identified by double-label immunohistochemistry. In these studies, another population of cyclin-positive cells was identified with a mesenchymal morphology that did not stain with any of the cell-specific antibodies used. Thus, these cells had similar properties as that of the PDGF-synthesizing MIC described in the in situ hybridization studies. It is tempting to speculate that some of the PDGF-positive MIC observed in plaques might be cyclin positive as well. This would place cell proliferation and growth factor expression at a site associated previously with plaque progression and intimal development. It is interesting to note that the plaque showing the highest intimal replication rate (4.5%) had an area of focal hemorrhage and thrombus organization. This suggests a link between cell proliferation and thrombus organization, but the sample size is much too small to make any firm conclusions from this report.

Although platelet deposition and release of growth factors associated with thrombus formation has been postulated to be important in initiating the cellular growth response after vascular injury, this hypothesis has been challenged by a recent report that platelet depletion with injections of a polyclonal antiplatelet antibody had little effect on the initiation of cell proliferation in the media after injury. Alternatively, fibroblast growth factors (FGF) have received increased attention as potential mitogens for the vascular wall cells since inhibition of basic fibroblast growth factor (bFGF) activity using injections of a specific monoclonal antibody against bFGF after BCA of the rat carotid artery prevented SMC proliferation at early time points after injury. Taken together, these studies suggest that FGF release from matrix and damaged cells may be more important than platelets in initiating the first wave of SMC proliferation. However, the antiplatelet experiments may be inconclusive because platelets were not completely depleted from the circulation throughout the postsurgical period despite repeated antibody injections. Moreover, the antiplatelet treatments reduced ultimate lesion size but the anti-FGF experiments did not. Clearly, more definitive studies are required to determine the contributions of platelets and FGF in lesion development.

There is considerable evidence that endothelial denudation in rats and rabbits induces SMC proliferation and development of a neointima. SMC proliferation begins in the media within 36 to 48 hours after injury, and after 1 week, SMCs migrate to form an intimal mass of actively proliferating cells. Proliferation continues in a single luminal layer of PDGF-synthesizing SMCs for as long as 3 months. Despite the acute accumulation of platelets on the exposed subendothelium after balloon denudation of normal vessels, fibrin- and red cell-rich thrombus is not typically observed after injury. However, if lesions develop and the vessel is reinjured, abundant fibrin/platelet thrombi form on the surface of the injured neointima. Thrombin activity has been associated with the exposed subendothelium after balloon denudation of normal vessels and remains elevated for up to 10 days after injury in the rat. Since thrombin stimulates smooth muscle proliferation, the finding of elevated thrombin generation at the site of continued smooth muscle cell proliferation implicated thrombin in the proliferative process.

The article by Sarembock et al suggests that by inhibiting the acute increase in thrombin activity after vascular injury, it is possible to affect ultimate lesion development. The article offers a tantalizing new lead into mechanisms underlying lesion development after injury, but many questions are left unanswered. What is the mechanism of action of hirudin in the present model? Cell proliferation studies are now critical to determine if hirudin modified lesion development by acting on smooth muscle proliferation or matrix deposition. Does hirudin work by reducing the first wave of smooth muscle proliferation in the media, is smooth muscle proliferation reduced throughout the postsurgical period, or has hirudin altered cell migration from the media to form the intima? Scanning electron microscopy studies are required to determine to what extent platelet deposition was modified by hirudin and for how long. Were platelets deposited on the injured surface after termination of the hirudin treatment, and does this mean that smooth muscle proliferation was inhibited in spite of platelet deposition and release? Fibrin and fibrin fragments are chemotactic for SMCs in vitro, and thrombin has been shown to stimulate growth factor release and SMC proliferation. Does
hirudin therefore modify lesion development through direct action on smooth muscle cells or does it act indirectly by altering the thrombotic response to injury? The recent cloning of the human thrombin receptor has made it possible to determine by Northern blots that thrombin receptors are synthesized by endothelial and smooth muscle cells, suggesting the potential for direct action of thrombin on these cells. Further analysis of the thrombin receptor's structure and its regulation will undoubtedly provide additional clues about how thrombin modifies cellular responses.

Given the evidence that 1) atherosclerotic plaques are procoagulant; 2) thrombi form after PTCA on the injured surface; and 3) a number of components comprising the forming thrombus may contribute to SMC proliferation, we must consider that thrombus formation may play a significant role in the development of proliferative lesions after vascular injury. It is tempting to speculate that hirudin modified vascular lesion development by directly inhibiting thrombin action on smooth muscle cell proliferation; clearly, this conclusion will have to await more definitive studies.

References

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J N Wilcox

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