See related article, pages 1141–1152

Tension in the Plaque: Hypoxia Modulates Metabolism in Atheroma

Peter Libby, Eduardo Folco

Investigators have long recognized low oxygen tension in the atherosclerotic plaque. The diffusion limitation for molecular oxygen through the relatively avascular center of the plaque decreases the supply of this gas. Inflammatory cells accumulate in the core of atheromata. These cells have high metabolic rates and correspondingly high oxygen consumption, increasing demand (Figure). Human and experimental atheromata have hypoxic regions. Animal studies have shown that hypoxia augments atherogenesis.[1,2] Recent work has highlighted the myriad mechanisms by which hypoxia may contribute to atheroma evolution and complication.[3,4]

Hypoxia Stimulates Plaque Angiogenesis

Pathologists have long appreciated the microvasculature of plaques. The rise of Judah Folkman's concept of tumor angiogenesis as a growth-promoting mechanism in malignancy stimulated parallel thinking in atherosclerosis research.[5] Plaque neovessels may stimulate lesion growth and provide a portal with a large surface area for penetration of inflammatory cells. Fragile neovessels in atheromata, as in the diabetic retina, may prove prone to hemorrhage. Extravasated erythrocytes furnish a local depot of cholesterol-rich red cell membranes and of heme, a source of iron—which is a catalyst for oxidative stress. Thrombosis in situ may elicit cycles of thrombin-mediated smooth-muscle cell (SMC) migration and proliferation and hence lesion growth. Thus the neovessels stimulated through the hypoxia-inducible factor (HIF)/vascular endothelial growth factor (VEGF) axis in response to hypoxia may promote intraplaque hemorrhage, lesion growth, recruitment of inflammatory cells, and oxidative stress.

Hypoxia Alters Glucose Metabolism in the Atherosclerotic Plaque

Through the regulation of glucose transporters (eg, GLUT-1) and enzymes that capture glucose within the cell (hexokinases), hypoxia augments glucose utilization by plaque cells— notably, the mononuclear phagocytes that abound in many lesions.[6] A shift to anaerobic glycolysis leads to lactate overproduction and lowers the pH prevailing in plaques. Hypoxia-driven increases in glucose uptake, incidentally, provide an opportunity to image plaque metabolism. Fluorodeoxyglucose (FdG), a tracer commonly used in tracking tumors using positron emission tomography, accumulates in some atherosclerotic plaques.[7] The avidity of atheromata for FdG uptake may provide a clinical window on some of the metabolic shifts associated with hypoxia.[6]

Could accelerated glucose utilization, and energy substrate depletion due to reduced delivery, alter plaque biology? Anerobic glycolysis yields much less adenosine triphosphate (ATP) per glucose molecule than does oxidative metabolism. Reduced ATP availability may promote mitochondrial and extramitochondrial pathways of apoptosis in the plaque. Furthermore, hypoxia causes an imbalance between electron transport and the intracellular O_2 concentration that leads to the production of reactive oxygen species and oxidative stress, further predisposing to cell death.[8]

Apoptosis of macrophages in atheromatous lesions favors formation of the "necrotic core" of the plaque, a structure associated with disruption of human atheromata and thrombosis. SMCs in the plaque manufacture most of the interstitial collagen that lends tensile strength to the plaque's protective fibrous cap; hence, SMC apoptosis can impair the cap's integrity.[9] Fracture of the fibrous cap causes most fatal acute myocardial infarctions in humans. Thus, sensitization of macrophages and SMCs to apoptosis by hypoxic conditions could contribute to the thrombotic complications of this disease.

Does Hypoxia Promote Plaque Proteolysis?

Proteolysis drives dissolution of the plaque extracellular matrix. Accelerated catabolism of extracellular matrix constituents likely contributes decisively to plaque evolution and complication. Outward remodeling (also called compensatory enlargement)—characteristic of arteries that harbor growing atheromata—requires reshaping of the extracellular matrix, a process that probably involves both elastolysis and collagenolysis. The penetration of microvessels from the adventitia into the plaque likewise requires digestion of extracellular matrix.[10] Excessive degradation of collagen may predispose toward plaque rupture by decreasing the collagen content of the plaque's protective fibrous cap. The catabolism of nonfibrillar collagen in the basement membrane of the arterial intima may set the stage for superficial erosion of the endothelial monolayer—another common mechanism of thrombosis complicating human atherosclerotic plaques—by altering the subendothelial matrix, thereby sensitizing these cells to death by anoikis.

From the Division of Cardiovascular Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA.

Correspondence to Peter Libby, MD, Division of Cardiovascular Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, 77 Avenue Louis Pasteur, NRB741, Boston, MA 02115. E-mail: plibby@rics.bwh.harvard.edu

(*Circ Res*. 2011;109:1100-1102.)

***Circulation Research* is available at http://circres.ahajournals.org**
DOI: 10.1161/RES.0b013e31823bdb84

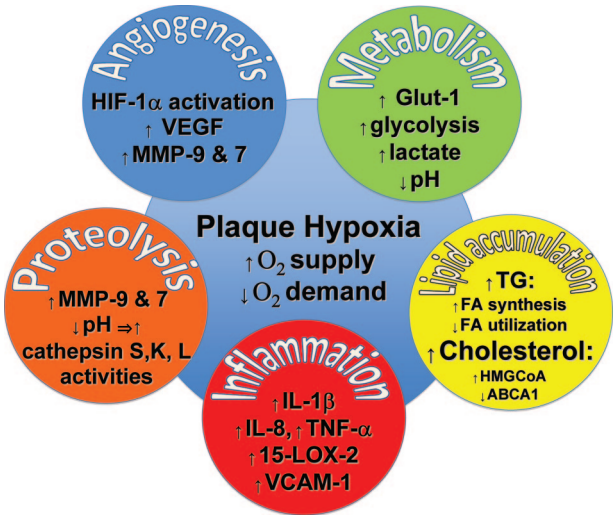

Figure. Hypoxia in atherogenesis. This figure illustrates selected examples of the manifold effects of hypoxia in atheromata; see text for explanation. 15-LOX-2 indicates 15-lipoxygenase-2; ABCA1, ATP-binding cassette A 1; FA, fatty acid; GLUT-1, glucose transporter 1; HIF-1α, hypoxia-inducible factor 1α; HMG-CoA, hydroxymethylglutaryl coenzyme A; IL, interleukin; MMP, matrix metalloproteinase; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor.

Hypoxia may regulate the enzymes involved in catabolism of the plaque's extracellular matrix in several ways. Hypoxic conditions may augment the activity of matrix metalloproteinases (MMPs), a family that includes interstitial collagenases that weaken the fibrous cap and gelatinases capable of catabolizing nonfibrillar collagen, to which endothelial cells adhere.[11–13] Hypoxia-induced MMP-7 may participate critically in atherothrombosis. In addition to directly contributing to extracellular matrix remodeling, this metalloproteinase can elicit proatherogenic molecules such as tumor necrosis factor α (TNF-α),[14] and promotes thrombogenicity by degrading tissue factor pathway inhibitor.[15] In a recently recognized novel twist, MMP-14 can augment HIF-1 activity by a *non*-proteolytic mechanism and increase macrophage ATP production, simulating hypoxic alterations in glucose metabolism.[16]

In addition, the drop in pH in hypoxic portions of plaques in lesions favors the activity of lysosomal hydrolases.[17] Notably, cysteinyl elastases—such as cathepsins S, K, and L—localize in plaques and contribute to lesion evolution.[18] These potent elastases may participate in remodeling of arteries during atherogenesis, among other functions.[8] Thus hypoxic regulation of proteolytic activity may have multiple consequences for plaque evolution and complication.

Hypoxic Conditions May Incite Inflammation in Plaques

Hypoxia can foster the formation of proinflammatory cytokines and leukotrienes, and activate Akt (Figure).[3,12] Ultimately, hypoxia and inflammation conspire to promote the evolution and clinical complications of atherosclerosis.

Lipid Accumulation

Mononuclear phagocytes subjected to hypoxia accumulate triglyceride, due to increased production of, and from reduced oxidation of, fatty acids.[3,19] Augmented expression of stearoyl-coenzyme A desaturase (SCD-1) may promote fatty acid synthesis in hypoxic mononuclear phagocytes. In this issue of *Circulation Research*, Parathath and colleagues show that hypoxic conditions augment cellular content of sterols as well as triglycerides. They implicate both increased production due to augmented hydroxymethylglutaryl coenzyme A (HMG-CoA) expression and decreased efflux mediated by ATP-binding cassette A (ABCA1) function.[20] Thus, hypoxia modulates the metabolism of both triglycerides and sterols—lipids that accumulate in macrophage foam cells, a hallmark of atheromata.

Implications of Plaque Hypoxia

Increased recognition of the low oxygen tension in regions of atheromata and its metabolic consequences has considerable implications for contemporary atherosclerosis research. In vitro experiments indubitably have advanced the understanding of mechanisms relevant to atherogenesis. Yet, most studies cultivate SMCs and macrophages under normoxic conditions. Our usual laboratory culture conditions strive to buffer the pH to maintain neutrality. Normoxia and pH 7.4 represent conditions far afield from those found in regions of the atheroma. Moreover, much contemporary experimental work in atherosclerosis relies on the use of mice. Due to their smaller size, mouse lesions may harbor less hypoxia than their human counterparts. While exceedingly informative, studies of cultured cells and of mouse atheromata should be considered in light of these important differences with conditions pertaining to human plaques.

Increased recognition of plaque hypoxia also has some pathophysiological implications, beyond these technical experimental points. The great German biochemist Otto Warburg described overutilization of glucose by cancer cells and constructed a unified theory of cancer related to some of the metabolic consequences of hypoxia. Warburg's unitary view vastly oversimplified the complex and multifactorial diseases lumped together as "cancer." Our concepts of the pathogenesis of atherosclerosis have likewise witnessed similar cycles of enthusiasm for specific mechanisms: bland lipid storage, mechanical injury, neoplastic-like SMC proliferation, oxidative stress, and inflammation. Hypoxia now garners recognition as a modulator of mechanisms that drive atherogenesis and its clinical consequences. Although Warburg's scientific insight stands, his monomaniacal view of cancer has fallen. We need to recognize that no one instigator or pathway explains atherogenesis in its full complexity. We stand to learn more about the disease, and have a greater chance of mastering it, if we appreciate its multifactorial mechanisms, including hypoxia.

References

1. Nakano D, Hayashi T, Tazawa N, Yamashita C, Inamoto S, Okuda N, Mori T, Sohmiya K, Kitaura Y, Okada Y, Matsumura Y. Chronic hypoxia accelerates the progression of atherosclerosis in apolipoprotein E-knockout mice. *Hypertens Res*. 2005;28:837–845.
2. Jun J, Reinke C, Bedja D, Berkowitz D, Bevans-Fonti S, Li J, Barouch LA, Gabrielson K, Polotsky VY. Effect of intermittent hypoxia on atherosclerosis in apolipoprotein E-deficient mice. *Atherosclerosis*. 2010; 209:381–386.

3. Hulten LM, Levin M. The role of hypoxia in atherosclerosis. *Curr Opin Lipidol*. 2009;20:409–414.
4. Sluimer JC, Daemen MJ. Novel concepts in atherogenesis: angiogenesis and hypoxia in atherosclerosis. *J Pathol*. 2009;218:7–29.
5. Moulton KS, Heller E, Konerding MA, Flynn E, Palinski W, Folkman J. Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E-deficient mice [see comments]. *Circulation*. 1999;99:1726–1732.
6. Folco EJ, Sheikine Y, Rocha VZ, Christen T, Shvartz E, Sukhova GK, Di Carli MF, Libby P. Hypoxia but not inflammation augments glucose uptake in human macrophages. Implications for imaging atherosclerosis with FdG-PET. *J Am Coll Cardiol*. 2011;58:603–614.
7. Fayad ZA, Mani V, Woodward M, Kallend D, Abt M, Burgess T, Fuster V, Ballantyne CM, Stein EA, Tardif JC, Rudd JH, Farkouh ME, Tawakol A. Safety and efficacy of dalcetrapib on atherosclerotic disease using novel non-invasive multimodality imaging (dal-PLAQUE): a randomised clinical trial. *Lancet.* 2011.
8. Semenza GL. Oxygen-dependent regulation of mitochondrial respiration by hypoxia-inducible factor 1. *Biochem J*. 2007;405:1–9.
9. Geng Y-J, Libby P. Evidence for apoptosis in advanced human atheroma. Co-localization with interleukin-1 b-converting enzyme. *Am J Pathol*. 1995;147:251–266.
10. Libby P, Schonbeck U. Drilling for oxygen: angiogenesis involves proteolysis of the extracellular matrix. *Circ Res*. 2001;89:195–197.
11. Kolev K, Skopal J, Simon L, Csonka E, Machovich R, Nagy Z. Matrix metalloproteinase-9 expression in post-hypoxic human brain capillary endothelial cells: H2O2 as a trigger and NF-kappaB as a signal transducer. *Thromb Haemost*. 2003;90:528–537.
12. Deguchi JO, Yamazaki H, Aikawa E, Aikawa M. Chronic hypoxia activates the Akt and beta-catenin pathways in human macrophages. *Arterioscler Thromb Vasc Biol*. 2009;29:1664–1670.
13. Burke B, Giannoudis A, Corke KP, Gill D, Wells M, Ziegler-Heitbrock L, Lewis CE. Hypoxia-induced gene expression in human macrophages: implications for ischemic tissues and hypoxia-regulated gene therapy. *Am J Pathol*. 2003;163:1233–1243.
14. Gearing AJ, Beckett P, Christodoulou M, Churchill M, Clements JM, Crimmin M, Davidson AH, Drummond AH, Galloway WA, Gilbert R, Gordon JL, Leber TM, Mangan M, Miller K, Nayee P, Owen K, Patel S, Thomas W, Wells G, Wood LM, Woolley K. Matrix metalloproteinases and processing of pro-TNF-alpha. *J Leukoc Biol*. 1995;57:774–777.
15. Belaaouaj AA, Li A, Wun TC, Welgus HG, Shapiro SD. Matrix metalloproteinases cleave tissue factor pathway inhibitor. Effects on coagulation. *J Biol Chem*. 2000;275:27123–27128.
16. Sakamoto T, Seiki M. A membrane protease regulates energy production in macrophages by activating hypoxia-inducible factor-1 via a non-proteolytic mechanism. *J Biol Chem*. 2010;285:29951–29964.
17. Levin M, Leppanen O, Evaldsson M, Wiklund O, Bondjers G, Bjornheden T. Mapping of ATP, glucose, glycogen, and lactate concentrations within the arterial wall. *Arterioscler Thromb Vasc Biol*. 2003;23:1801–1807.
18. Liu J, Sukhova GK, Sun JS, Xu WH, Libby P, Shi GP. Lysosomal cysteine proteases in atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2004;24:1359–1366.
19. Bostrom P, Magnusson B, Svensson PA, Wiklund O, Boren J, Carlsson LM, Stahlman M, Olofsson SO, Hulten LM. Hypoxia converts human macrophages into triglyceride-loaded foam cells. *Arterioscler Thromb Vasc Biol*. 2006;26:1871–1876.
20. Parathath S, Mick SL, Feig JE, Joaquin V, Grauer L, Habiel DM, Gassmann M, Gardner LB, Fisher EA. Hypoxia is present in murine atherosclerotic plaques and has multiple adverse effects on macrophage lipid metabolism. *Circ Res.* 2011;109:1141–1152.