Oxidative Stress and Coronary Plaque Stability

Keith M. Channon

Increased production of reactive oxygen species (ROS) in the vascular wall is a characteristic feature of disease states, including atherosclerosis, diabetes and hypertension. ROS, such as superoxide, reduce nitric oxide bioactivity by scavenging and cause oxidation of lipids and target proteins. In addition, recent work has revealed that ROS mediate a wide range of pathological processes in the endothelium, smooth muscle cells, and inflammatory cells. ROS are generated by enzyme systems present in cells in the vascular wall, including NAD(P)H oxidase, xanthine oxidase, and nitric oxide synthase. The activities and levels of these enzyme systems are increased in association with vascular disease risk factors and in vascular disease states in which oxidative stress is prominent, for example, in diabetes and atherosclerosis.

The NAD(P)H oxidases appear to be particularly important sources of ROS production in blood vessels, where they are constitutively active, producing relatively low levels of ROS under basal conditions, but generating higher levels of oxidants in response to stimuli such as growth factors and cytokines. These factors are consistent with a role for nonphagocytic NAD(P)H oxidases in cellular signaling rather than the high-level burst activity characteristic of the phagocyte NAD(P)H oxidase. The NAD(P)H oxidases are multimeric enzyme systems composed of plasma membrane–associated–proteins as well as cytosolic factors. In the phagocytic-type NAD(P)H oxidase, the plasma membrane–associated–proteins gp91phox and p22phox compose the flavocytochrome b558 complex, which forms the catalytic subunit of the oxidase. The cytosolic subunits, including p47phox, p67phox, and the G-protein Rac, regulate oxidative function.

Azumi and colleagues, in this issue of *Arteriosclerosis, Thrombosis and Vascular Biology*, have made important additions to our understanding of the role of ROS in human coronary artery disease. They studied directional coronary atherectomy (DCA) specimens taken from 36 patients with stable or unstable angina pectoris undergoing percutaneous coronary intervention. The cellular composition of these plaque fragments was examined by using immunofluorescence and related to the magnitude and cellular sources of ROS production, visualized by using the superoxide-sensitive fluorescent probe, dihydroethidium (DHE). They report that both ROS production and the presence of oxidized LDL (Ox-LDL) are spatially associated with the p22phox subunit of the NAD(P)H oxidase, directly implicating this enzyme system in ROS generation and oxidative modification of target molecules in human coronary plaques. Indeed, in earlier work, Azumi et al showed that total p22phox protein levels were increased in atherosclerotic coronary arteries. In the current study, they now demonstrate that these markers of ROS production were markedly increased in macrophage-rich plaques, that were more likely to originate from patients presenting with unstable rather than stable angina pectoris. Plaques from patients with stable angina tended to have lower ROS production, fewer macrophages and a greater proportion of smooth muscle cells. However, unstable plaques that contained a substantial proportion of smooth muscle cells also showed an additional increase in ROS production in these cells, suggesting up-regulation of ROS production in both smooth muscle cells (SMCs) and macrophages in unstable plaques. These observations are supported by studies of atherosclerotic lesions from human aorta, where activated intimal SMCs (but not medial SMCs) and macrophages expressed high levels of NAD(P)H oxidase subunits, and this increase was most striking in macrophage-derived foam cells.

These findings are important because they extend our understanding of the biology of plaque stability versus instability and suggest that ROS production in human coronary artery plaques may play a role in regulating plaque stability and in mediating the occurrence of acute coronary syndromes. What are the potential mechanisms that relate ROS production to plaque stability? At the very least, increased ROS production appears to be a marker of unstable plaques, due to the higher proportion of active macrophages present in these lesions. However, several other biologically plausible mechanisms suggest that ROS production may play more direct roles in modulating plaque stability. Superoxide, and peroxyxinitrite formed by the interaction between superoxide and nitric oxide, are proinflammatory radicals, leading to activation of redox-sensitive transcription factors such as nuclear factor κB and activating protein-1 in endothelial and SMCs, and in macrophages. Superoxide induces expression of matrix-degrading proteases, including MMP-2 and MMP-9 in foam cells that directly contribute to plaque instability. The observation by Azumi et al of increased Ox-LDL immunofluorescence in association with DHE fluorescence underscores that ROS production promotes formation of Ox-LDL, allowing macrophage scavenger receptor-mediated uptake of Ox-LDL, leading to activated foam cell formation. Ox-LDL has additional effects on smooth muscle and endothelial cell apoptosis that could lead to endothelial erosion and loss of SMCs, increasing plaque vulnerability.

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The interesting observation of increased ROS production in smooth muscle cells in plaques from patients with unstable angina raises new questions about the role of ROS signaling in SMC biology in the atherosclerotic plaque. The unstable, lipid-rich plaque is characterized by a proportionately lower SMC component and increased SMC apoptosis. In contrast, fibrocellular lesions or the intimal response to balloon or stent injury are SMC-rich, causing luminal stenosis but not acute events related to lesion instability. However, SMC phenotype is an important additional consideration. Activated, “de-differentiated” SMCs produce more superoxide and express higher levels of NAD(P)H oxidase components. Correspondingly, NAD(P)H oxidase-derived ROS play important roles in SMC proliferation. Cytokines such as IFN and growth factors, implicated in modulating plaque stability, have marked effects on NAD(P)H oxidase subunit expression and ROS production. Furthermore, the recent identification of SMC homologs of gp91phox (Nox2), Nox1 and Nox4, points to increased complexity of NAD(P)H oxidase regulation in SMCs. Increased or decreased Nox1 expression directly alters cell proliferation in culture, and treatment of SMCs with angiotensin II upregulates Nox1 while downregulating Nox4, suggesting reciprocal regulation of these proteins in relation to SMC growth. After arterial balloon injury in the rat, increased superoxide production coincides with activated SMCs and fibroblasts, in association with intimal hyperplasia. Interestingly, expression of Nox1, gp91phox, and p22phox is elevated early after balloon denudation, suggesting that NAD(P)H oxidase activity is rapidly induced after vascular injury in vivo, in both SMCs and fibroblasts. In contrast, Nox4 expression increases later after balloon injury, coinciding with a marked reduction in the rate of SMC proliferation. Upregulation of Nox4 in the arterial wall in vivo could attenuate SMC proliferation after vascular injury, thus limiting the “repair” response and may also contribute to induction of apoptosis of neointimal cells that is observed late after balloon injury. Thus, regulation of both ROS production and the expression of NAD(P)H oxidase components is directly relevant to the biological role of SMCs in determining plaque stability versus instability.

In human coronary artery segments retrieved from cardiac transplant recipients, Sorescu et al also observed striking increases in both ROS production and NAD(P)H oxidase subunits p22phox and gp91phox (Nox2) expression in association with increasing severity of atherosclerotic plaque. The presence of Nox2 was strongly associated with plaque macrophage content, whereas Nox4 was associated with SMCs and was most abundant in advanced fibrocellular plaques, but not in complex plaques with features of instability. Most interestingly for plaque stability, the shoulder region of plaques was a particularly intense area of ROS production, in association with p22phox and Nox2 expression, implicating NAD(P)H oxidase-derived ROS in plaque rupture. A plausible mechanistic link with plaque stability is supported by the observation that HMG CoA-reductase inhibitors (statins), known to stabilize plaques and reduce plaque events, have direct effects on NAD(P)H oxidase activity through inhibition of the Rac G-protein involved enzyme activation.

The important new observations of Azumi et al, taken together with these other studies, begin to paint a clearer picture of the role of ROS production by NAD(P)H oxidases in plaque biology and their relationship to clinical coronary syndromes. Future studies need to investigate more directly how molecular regulation of NAD(P)H oxidase components is related to the roles of macrophage and SMCs in mediating plaque stability. ROS production by both macrophages and SMCs and the subsequent effects of ROS on SMC phenotype, growth, and apoptosis may provide novel potential targets for modifying plaque stability in human atherosclerosis.

References
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