Editorial

Quest for Fire

Seeking the Source of Pathogenic Oxygen Radicals in Atherosclerosis

David Schultz, David G. Harrison

Abstract—There is an accumulating body of evidence that atherosclerosis is either caused by or accompanied by oxidative events in the vessel wall. These oxidative events have been implicated in proatherogenic modification of proteins, alteration of gene expression, promotion of inflammation, remodeling of vessels, and perturbations of vascular tone. This body of literature has led to a dogma that oxidation is a prerequisite for the atherosclerotic process. In particular, oxidation of lipoproteins by activated macrophages in the subintimal space has been postulated to be an important early step in the atherosclerotic process. (Arterioscler Thromb Vasc Biol. 2000;20:1412-1413.)

Key Words: editorials ■ reactive oxygen species ■ superoxide ■ atherosclerosis ■ NADPH oxidases

In macrophages, the predominant source of reactive oxygen species (ROS) is the NADPH oxidase. This enzyme system was first characterized in the neutrophil and is composed of at least 5 subunits: 2 cytosolic components, p47phox and p67phox; 2 membrane-bound components, a small, nonglycosylated p22phox and a larger, glycosylated gp91phox (which together compose the cytochrome b558); and a low-molecular-weight G protein, rac-1.1 Numerous mutations in these subunits have been described in humans with chronic granulomatous disease (CGD).2 Mice lacking gp91phox have been created and have a phenotype characteristic of CGD. Because this enzyme system is such a potent source of ROS, it has been strongly suspected to be involved in lipid oxidation and the initiation of atherosclerosis.

The article by Kirk et al3 represents a significant advance in our understanding of atherosclerosis. The study clearly shows that the macrophage NADPH oxidase is not critical in the development of atherosclerosis. Does this mean that ROS do not have a role in atherosclerosis? We cannot push our conclusions that far. The data prove that superoxide generated from a gp91phox-containing oxidase is not important in atherosclerosis, but they do not exclude a role for other sources of radicals. Importantly, the authors did not show that vessels from the gp91phox-deficient animals indeed had reduced superoxide production. Quantification of vascular superoxide production by using techniques such as lucigenin-enhanced chemiluminescence or electron spin resonance would have provided invaluable information on whether the gp91phox knockout or the double knockout altered vascular superoxide production. Measurements of parameters of lipid peroxidation, although difficult in these small animals, would have also been helpful. Such parameters include measurements of conjugated dienes, thiobarbituric acid–reactive substances, antibodies against oxidized lipids, or oxidized protein epitopes in either the blood or the vessel wall. In the absence of these measurements, it is impossible to conclude that oxidative processes can be excluded as having a role in atherosclerosis.

Furthermore, the study by Kirk et al3 does not definitively exclude an NADPH-like enzyme as having a role in atherosclerosis. Recently, it has become evident that nonphagocytic cells in the vessel wall, including endothelial cells, vascular smooth muscle cells, and cells of the adventitia, can produce ROS and seem to contain enzyme systems similar to the NADPH oxidase of neutrophils.4 These “vascular oxidases” may utilize different subunits than do the neutrophil oxidases. In isolated endothelial cells, reverse transcriptase–polymerase chain reaction has demonstrated the expression of p22phox, gp91phox, p47phox, and p67phox, but Northern blot analysis was able to detect significant amounts of only p22phox mRNA. Immunoperoxidase staining revealed the presence of p47phox and p67phox protein. Although no antibodies were available to detect the 2 membrane-bound components, hemoglobin spectroscopy was unable to detect a cytochrome b558 signal.5 In vascular smooth muscle, p22phox has been cloned and is
produce large amounts of ROS. In this regard, Miller et al.\(^8\)
and their vascular smooth muscle cells could continue to
MOX-1 would be present in the gp91phox-deficient mice,
gp91phox in vascular smooth muscle cells. Importantly,
more alternative pathways. Lipoprotein oxidation is critically
enzymatic pathway often leads to compensation by 1 or
edly been hammered home from studies of knockout mice is
alternative explanations for the lack of an effect of the
gp91phox knockout on atherosclerosis development. As dis-
filling the bill.
In the previous several paragraphs, we have offered several
alternative enzyme source of radicals is important in the oxidation of lipids. Several
different reactive oxygen intermediates can be shown in vitro
to initiate lipid oxidation and peroxidation. These include
superoxide, hydrogen peroxide, peroxynitrite, hypchlorous
acid, and the hydroxyl radical. In the case of severe hyper-
cholesterolemia, as produced in the cholesterol-fed mice or
the apoE-deficient mice studied by Kirk et al.,\(^3\) it is quite
likely that many different ROS, derived from many different
sources, can react with the abundant lipoproteins that accu-
mulate in the vessel wall. Alternatively, lipooxygenase en-
zymes react with esterified fatty acids to directly form the
lipid alkoxyl radical (LO). This lipid radical can react with
molecular oxygen to form a lipid alkylperoxyl radical (LOO). In this manner, lipoxygenase may initiate lipid
peroxidation without forming superoxide or any of superox-
dize’s reactive products. Of substantial importance, a 12-li-
poxygenase–deficient mouse has been created and crossed
with the apoE-deficient mouse. Mice lacking both the apoE
and the lipoxygenase gene had a dramatic reduction in
atherosclerotic lesions compared with mice lacking the apoE
gene only.\(^9\) Thus, it is possible that in the normal situation,
superoxide (and other radicals) derived from the phagocytic
NADPH oxidase contributes to lipid oxidation, but when this
enzyme is absent, other sources are perfectly capable of
filling the bill.
In the previous several paragraphs, we have offered several alterna-
second alternative explanations for the lack of an effect of the
gp91phox knockout on atherosclerosis development. As dis-
cussed, 1 viable alternative is that lipoxygenase enzymes or
other enzymes are far more important in lipid peroxidation
than is the NADPH oxidase. Another lesson that has repeat-
etly been hammered home from studies of knockout mice is
that substantial redundancy exists in biology, and deletion of
1 enzymatic pathway often leads to compensation by 1 or
more alternative pathways. Lipoprotein oxidation is critically

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