How Vascular NAD(P)H Oxidase Activity and Nox Isoform Expression are Regulated

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It is well known that endothelial dysfunction is a systemic disorder and a key variable in the initiation and progression of atherosclerosis and its complications.1 Most risk factors that are related to atherosclerosis and cardiovascular morbidity and mortality, including hypercholesterolemia, hypertension, cigarette smoking, and diabetes mellitus, are found to be associated with endothelial dysfunction. Some studies have shown that the risk for developing endothelial dysfunction increases with the number of risk factors present in an individual. Endothelial dysfunction is not confined to the coronary arteries but rather represents a systemic disorder that also affects peripheral vascular beds, including both conduit arteries and small resistance vessels in the extremities. Therefore, a uniform underlying mechanism(s) is suggested to account for the impaired endothelial function.

Recent studies have demonstrated that increased production of reactive oxygen species in human blood vessels is associated with endothelial dysfunction. The major source of superoxide anion in the vasculature is NAD(P)H oxidase family of enzymes. NAD(P)H oxidases are present in the vascular cells including endothelial cells, smooth muscle cells, fibroblasts, and macrophages.2 Vascular NAD(P)H oxidase is a multisubunit enzyme complex that differs structurally and biochemically from the phagocytic NAD(P)H oxidase. This superoxide-generating enzyme includes the membrane-bound flavocytochrome b558 formed by gp 91 phox and p22 phox and the cytosolic proteins p47 phox, p67 phox, and Rac. Recently, novel gp 91 phox (Nox2) homologues, termed Nox 1, Nox 3, Nox 4, and Nox 5, were identified in nonphagocytic cells, including Nox1 and Nox 4 in the vasculature.3 The simultaneous presence of multiple Nox proteins are demonstrated in one cell type. In vascular smooth muscle cells, gp 91 phox is expressed at very low levels, and Nox 1 and Nox 4 are coexpressed at much higher levels. Nox 4 as well as Nox 2 is expressed in vascular endothelial cells. Each Nox is involved in superoxide production. In cells that express more than one gp 91 phox homologue, it is possible that each Nox protein serves a specific biological function and that different intracellular localization of each Nox protein determines the distinct role of each Nox protein in cell functions. The localization of Nox 1 in caveolae could be involved in cell growth-promoting actions by angiotensin II type 1 receptors. The localization of Nox 4 in focal adhesions and in the nucleus could be associated with integrin-linked signaling (eg, stretch) and aspects of gene expression (eg, growth, differentiation, inflammatory reactions, senescence, and apoptosis), respectively.4 Nox 1 is inducible and upregulated by growth factors and hormones, whereas Nox 4 is downregulated by these agonists. Demonstration of the nox response to vascular injury indicates that the expression of nox 1 and nox 4 in the vascular wall is temporary and spatially regulated in vivo.5 Indeed, early elevations of nox1 and p22 phox mRNA are observed with concomitant elevation in superoxide level, and a late increase in nox 4 expression also occurs.

There are several human studies examining the relationship between NAD(P)H oxidase subunit expression and atherosclerosis. Azumi et al examined the expression of p22 phox in atherosclerotic and nonatherosclerotic coronary arteries and found a significant increase in p22 phox expression across the vessel wall in diseased arteries.6 As atherosclerosis progresses, p22 phox expression increases. Azumi et al have extended their findings by demonstrating that there is a significant correlation between generation of reactive oxygen species and the expression of p22 phox or oxidized LDL in directional coronary arterectomy specimens from patients with angina pectoris.7 Sorescu et al examined Nox family protein expression in coronary arteries from explanted hearts and found that p22 phox is expressed at higher levels in all cell types, whereas gp 91 phox is most abundant in macrophages.8 Smooth muscle cells and fibroblasts have much lower levels of gp 91 phox, Nox 4 is abundantly expressed in nonphagocytic cells, but Nox 1 levels are low in all cell types.

In the present issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Guzik et al report that the molecular composition of the NAD(P)H oxidase differs in saphenous veins and internal mammary arteries from the same patient undergoing coronary bypass surgery; saphenous veins express more nox 2 and p22 phox, whereas the relative expression of nox 4 is greater in arteries.9 However, there are strong correlations between p22 phox and nox4 mRNA expression and between superoxide production, NAD(P)H oxidase activity, and endothelial function in arteries and veins.

The new finding in this study is the importance of systemic unifying factors on the molecular regulation of the NAD(P)H oxidases in human vascular disease (Figure). In a previous study, the authors found that clinical risk factors such as...
hypercholesterolemia and diabetes are most strongly associated with NAD(P)H oxidase activity in human saphenous vein.\(^{10}\)

Genetic predisposition, such as polymorphisms in the NAD(P)H oxidase subunit genes, and other yet-unknown factors could also affect endothelial function and oxidative stress in a systemic fashion. There are two limitations in this study. The authors analyzed expression of NAD(P)H oxidase subunit mRNA using quantitative fluorescent RT-PCR method. It is clear that mRNA levels do not necessarily correspond to NAD(P)H oxidase activity. The study in vitro does not correctly reflect in vivo circumstances, as differences in hemodynamic force affect NAD(P)H oxidase subunit expression and enzyme activity.

The treatment with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), ACE inhibitors, and angiotensin II type 1 receptor antagonists have been shown to block the production of vascular reactive oxygen species and improve endothelial function. Recently, Wagner et al reported that statins suppress the assembly of NADPH oxidase subunit.\(^{11}\) It is interesting to examine the relationship between endothelial function, superoxide generation, and NAD(P)H oxidase activity/expression after treatment with statins.

### References

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