Over the past two decades, the number of scientific publications addressing the role of oxidative stress in physiology and pathophysiology has increased exponentially. Almost all cardiovascular disease states including hypertension, hyperlipidemia, diabetes, arteriosclerosis, unstable angina, vasculitis and myocarditis, restenosis as well as ischemia/reperfusion have been linked to an enhanced generation of oxygen-derived free radicals. Consequently, oxidative stress is generally considered to be a major progression factor for cardiovascular disease, despite the fact that the majority of the large prospective, controlled trials have failed to uncover a beneficial effect of antioxidative treatment.

Therefore, one is left to wonder, whether an unspecific antioxidative radical scavenging approach is suitable to substantially lower oxidative stress and to affect disease progression. Indeed, individual sources of oxidative stress and the contribution of these enzymes to disease progression have to be identified. This approach may ultimately lead to the development of "specific" inhibitors of oxidative stress to target the intracellular source of free radical generation.

An important source of oxygen-derived radical generation are the NADPH oxidases, ubiquitous to all vascular cells. These enzymes, which in their subunit composition are either similar or even identical to the leukocyte NADPH oxidase can be induced and activated by many factors involved in the initiation and progression of cardiovascular disease such as thrombin, tumor necrosis factor-α, platelet-derived growth factor, and proatherosclerotic lipids such as lysophosphatidyl choline and Lp(a). The best characterized stimulus for NADPH oxidase activation and induction is angiotensin II, and experiments utilizing mice lacking different NADPH oxidase subunits have demonstrated that the oxidases plays a central role in angiotensin II–induced hypertension and hypertrophy.

In this issue of Circulation Research, Jacobson et al report that inhibition of vascular NADPH oxidases suppresses angioplasty-induced neointimal hyperplasia in the carotid artery of rats. By using the specific peptide inhibitor gp91ds-tat applied over an extended observation period, this study is one of the few clearly demonstrating involvement of NADPH oxidases in a specific disease state. Moreover, by demonstrating that this peptide inhibitor not only prevents neointima formation, inhibits stretch-induced superoxide anion release from distended vessels, as well as peroxynitrite formation after angioplasty, the authors have forged a clear link between neointima formation and NADPH oxidase–dependent radical formation.

Numerous studies have previously suggested that stretch, neointima formation, and restenosis are all associated with an increased vascular superoxide anion release. But although an increased expression and activity of NADPH oxidases has been documented previously in these studies, the lack of specific oxidase inhibitors has precluded mechanistic investigations. With the use of gp91ds-tat, a direct evidence for an involvement of NADPH oxidases has now been provided.

gp91ds-tat appears to be the only specific NADPH oxidase inhibitor currently available. This chimeric peptide consists of a tat site, derived from the tat peptide of the HIV virus, allowing uptake into the cell, and a fragment of gp91phox (Nox2), which has previously been shown to prevent the interaction of p47phox with the Nox subunits in cell-free preparations. As this p47phox-blocking peptide sequence is specific for the NADPH oxidases, it is likely that gp91ds-tat acts specifically on the oxidase, which makes it a unique tool for studying the involvement of the NADPH oxidase, particularly in vivo models. Several pharmacological, nonpeptide-based inhibitors of the oxidase are available, but the lack of specificity of these compounds has been a longstanding matter of concern (Figure 1).

Nevertheless, gp91ds-tat has some limitations. For example, it is not possible to differentiate between the Nox-containing oxidases as the peptide sequence is conserved in all vascular homologues of the oxidase. Moreover, the sensitization against the peptide will most probably lead to the formation of antibodies, limiting the treatment duration to a couple of weeks. Finally, the tat sequence of the peptide has been shown to cause side effects affecting cellular activity and signaling. Consequently, for chronic studies, specifically addressing the contribution of individual NADPH oxidase isoforms, additional molecular tools and compounds have to be developed.

An alternative approach is to use transgenic animals. Indeed, mice lacking the NADPH oxidase subunits p47phox and gp91phox (Nox2) have been used to demonstrate the involvement of the oxidase in angiotensin II–induced hypertension and hypertrophy as well as in VEGF-induced angiogenesis. In contrast, studies investigating the role of
the oxidase in the development of arteriosclerosis, using ApoE/NADPH oxidase subunit double knockout mice on a Western-type diet were negative15,16 or indicate that the oxidase is not the main progression factor for arteriosclerosis in ApoE−/− mice.17 Such reports contrast with those describing an increased expression of NADPH oxidase subunits as well as enhanced vascular superoxide anion formation in arteriosclerosis18–20 and highlight the need for novel approaches that differentially address the contribution of different sources of oxidative stress during cardiovascular disease. Indeed, for early stages of arteriosclerosis, particular in the setting of hyperlipidemia, xanthine oxidase,21 cytochrome P450 epoxygenases,22 and NO synthase isoforms23 might be of greater importance than NADPH oxidases (Figure 2).

Certainly, the present study addresses only one particular aspect of the role of NADPH oxidases in pathophysiology; the convincing demonstration of the effectiveness of gp91ds-tat peptide for inhibiting the enzyme in vivo, however, will spur on this field toward its goal of elucidating the contribution of oxygen-derived free radicals in vascular homeostasis and cardiovascular disease. Under physiological conditions, NADPH oxidases appear to be the primary source of vascular radical generation. Several conditions can further enhance NADPH oxidase–dependent radical formation and increase vascular xanthine oxidase activity as well as lead to the induction of cytochrome P450 epoxygenases. The NO synthase can also switch from an NO− to a superoxide anion–generating enzyme, if the essential cofactor tetrahydrobiopterin is oxidized.

Figure 1. Mechanism of action of NADPH oxidase inhibitors. Activation of the enzyme occurs after translocation of Rac and p47phox to the membrane-bound subunits Nox and p22phox. HMG-CoA reductase inhibitors (statins), as well as clostridium toxins, prevent Rac-mediated activation of the oxidase, whereas apocynin has been suggested to prevent translocation of p47phox by oxidizing SH groups. gp91ds-tat prevents the interaction of p47phox with Nox. Diphenylene iodonium (DPI) blocks the flavin domain of the oxidase, which is required for electron acceptance. Steroids, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, as well as inhibitors of protein kinase C (PKC) have been shown to prevent induction of the oxidase on stimulation with agonists.

Figure 2. Enzymatic sources of oxygen-derived free radical generation in the vasculature. Under physiological conditions, NADPH oxidases appear to be the primary source of vascular radical generation. Several conditions can further enhance NADPH oxidase–dependent radical formation and increase vascular xanthine oxidase activity as well as lead to the induction of cytochrome P450 epoxygenases. The NO synthase can also switch from an NO− to a superoxide anion–generating enzyme, if the essential cofactor tetrahydrobiopterin is oxidized. HMG-CoA inhibitors (statins), by depleting the cells of isoprenoids, prevent the integration of Rac in the plasma membrane, where it is involved in activating the oxidase.24 Consequently, it is tempting to speculate that some of the beneficial effects of statins and ACE inhibitors on vascular homeostasis and cardiovascular disease are a consequence of NADPH oxidase inhibition.

References

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**Key Words:** oxidative stress • NADPH oxidase • restenosis • neointima
A Radical Adventure: The Quest for Specific Functions and Inhibitors of Vascular NAPDH Oxidases

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Circ Res. 2003;92:583-585
doi: 10.1161/01.RES.0000066880.62205.B0
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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