A Radical Adventure
The Quest for Specific Functions and Inhibitors of Vascular NADPH Oxidases

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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.2

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.3 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 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.3

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.4 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,6 neointima formation,7 and restenosis8 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,7,8 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.9 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.10 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 hypertension11 and hypertrophy12,13 as well as in VEGF-induced angiogenesis.14 In contrast, studies investigating the role of

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Circulation Research is available at http://www.circresaha.org
DOI: 10.1161/01.RES.0000066880.62205.B0

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the oxidase in the development of arteriosclerosis, using ApoE/NADPH oxidase subunit double knockout mice on a Western-type diet were negative or indicate that the oxidase is not the main progression factor for arteriosclerosis in ApoE mice. Such reports contrast with those describing an increased expression of NADPH oxidase subunits as well as enhanced vascular superoxide anion formation in arteriosclerosis 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, cytochrome P450 epoxygenases, and NO synthase isoforms 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 may reveal vascular NADPH oxidases as a new target for drug development.

Regarding this aspect, it is noteworthy that two classes of compounds widely used in cardiovascular practice today can act as potent, although nonspecific inhibitors of NADPH oxidases. ACE inhibitors, by inhibiting angiotensin II formation, prevent the induction and activation of the NADPH oxidase on stimulation with agonists.


Key Words: oxidative stress, NADPH oxidase, restenosis, neointima
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Circ Res. 2003;92:583-585
doi: 10.1161/01.RES.00000066880.62205.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

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http://circres.ahajournals.org/content/92/6/583

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