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

Ralf P. Brandes

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 this purpose, and experiments utilizing mice lacking different NADPH oxidase isoforms, additional molecular tools and compounds have to be developed.

Moreover, the involvement of the oxidase in angiotensin II–induced hypertension and angioplasty-induced neointimal hyperplasia in the carotid artery of rats 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 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 the setting of hyperlipidemia, xanthine oxidase, cytochrome P450 epoxygenases, and NO synthase isoforms may be of greater importance than NADPH oxidases (Figure 2).

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.

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

10. Jia H, Lohr M, Jezequel S, Davis D, Shaikh S, Selwood D, Zachary I. Cysteine-rich and basic domain HIV-1 Tat peptides inhibit angiogenesis...


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

Circ Res. 2003;92:583-585
doi: 10.1161/01.RES.0000066880.62205.B0

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/92/6/583

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/