Inhaled nitric oxide is an elegantly simple therapy for idiopathic pulmonary hypertension. Breathing small amounts of nitric oxide directly activates guanylyl cyclase in pulmonary resistance vessels to counteract hypertension. In patients who respond to inhaled nitric oxide, either the endogenous synthesis of nitric oxide is not sufficient to overcome the hypertensive stress or nitric oxide is being inactivated too rapidly to act on guanylate cyclase. Certainly, both processes could be operating simultaneously.

Although nitric oxide has a reputation for being highly toxic, in reality the risk of toxicity with inhaled nitric oxide is minor because nitric oxide itself is unreactive with most biological molecules and the amounts administered are low enough to minimize the formation of nitrogen dioxide. Nitric oxide becomes toxic when converted to secondary reactive nitrogen species. Nitric oxide itself is swept away from the vasculature by rapid reactions with hemoglobin. In the high oxygen environment of the lung, nitric oxide will also not significantly inhibit mitochondrial respiration. By now, many patients have been breathing nitric oxide for weeks and even months without overt harm.

However, sudden termination of inhaled nitric oxide occasionally causes a potentially life-threatening hypertensive rebound, even when treated for a few hours. Hypertensive rebound can occur in individuals who showed no initial vasodilation in response to nitric oxide. In this issue of *Circulation Research*, Wedgwood et al \(^1\) have shown that endothelin, the most potent vasoconstrictor known, may contribute to rebound hypertension by inducing superoxide synthesis in the pulmonary vasculature. The increased flux of superoxide will react with nitric oxide to form peroxynitrite. The authors further show that endothelial nitric oxide synthase becomes nitrated on tyrosine, and the endogenous synthesis of nitric oxide by endothelium is decreased. Misfolding of endothelial nitric oxide synthase becomes nitrated on tyrosine, and the endogenous synthesis of nitric oxide by endothelium is decreased. Misfolding of endothelial nitric oxide synthase can cause the enzyme itself to produce superoxide.\(^2-4\) Activation of these pathways could not only contribute to rebound hypertension but could also be a contributing factor for the failure of nitric oxide therapy to affect pulmonary hypertension in unresponsive patients.

Peroxynitrite is formed by the diffusion-limited radical-radical reaction between superoxide and nitric oxide. Diffusion-limited is simply a chemist’s jargon implying that every time nitric oxide bumps into superoxide (which is controlled by diffusion), the two produce peroxyxynitrite. Because nitric oxide is 1000 times smaller than copper, zinc superoxide dismutase (SOD), it diffuses faster and therefore reacts with superoxide at least 10 times faster than SOD can possibly scavenge superoxide.\(^5,6\) Because of this competitive advantage, a substantial fraction of any superoxide produced in lung will produce peroxyxynitrite when micromolar nitric oxide is being inhaled.

Peroxynitrite is itself a strong oxidant and when protonated will produce the strongly oxidizing radicals hydroxyl radical and nitrogen dioxide.\(^7,8\) Peroxyxynitrite reacts rapidly with carbon dioxide to form nitrogen dioxide and bicarbonate radical, which can be even more damaging than the overly promiscuous hydroxyl radical. Although peroxyxynitrite is thermodynamically a potent oxidant, it tends to react rather selectively with many biological molecules. The surprising result is that peroxyxynitrite can activate a variety of signaling pathways, which tend to be proinflammatory and hypertensive (see Figure).

Peroxyxynitrite promotes the inflammatory synthesis of prostaglandin. The conjugate acid of peroxyxynitrite (ONOOH) plays an important role in vivo in the activation of cyclooxygenase by providing the peroxide tone necessary for enzyme activity. The non–heme iron in cyclooxygenase must initially be oxidized to an unstable and reactive ferryl state to be able to oxidize arachidonic acid. Marnett and colleagues have shown that peroxyxynitrite is the major peroxide responsible for activating cyclooxygenase in vivo.\(^9,10\) The product of cyclooxygenase, PGH\(_2\), is usually converted by prostacyclin synthase to the vasodilatory and antithrombotic prostaglandin (PGI\(_2\)). Curiously, prostacyclin synthase is itself exceptionally susceptible to attack by peroxyxynitrite, which appears to inactivate the enzyme by nitration of a critical tyrosine residue.\(^11,12\)

Accumulating PGH\(_2\) from cyclooxygenase can activate thromboxane receptors with similar potency as thromboxane itself. Thus, the reaction of superoxide with nitric oxide in the vasculature can help subvert the principal vasodilating mechanisms of endothelium into becoming strongly vasoconstricting.

Peroxyxynitrite induces longer-lasting changes in the vasculature and the immune system by deactivating anti-inflammatory agents as well as activating other stress-related

---

\(\text{OONO}^\text{−}\) \text{OONO}^\text{−} Rebounding From Nitric Oxide

Joseph S. Beckman

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

From the Linus Pauling Institute, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Ore.

Correspondence to Joseph S. Beckman, Ava Helen Pauling Chair, Linus Pauling Institute, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331. E-mail Joe.Beckman@orst.edu


© 2001 American Heart Association, Inc.
Inhaled nitric oxide will rapidly diffuse through the pulmonary epithelium to activate guanylyl cyclase in vascular smooth muscle. It will further diffuse through endothelium to react with hemoglobin, principally forming met-hemoglobin and nitrate. However, when tissues produce superoxide, some of the nitric oxide will be converted to peroxynitrite that will cause some tissue damage. Certain proinflammatory pathways are particularly reactive to peroxynitrite. Consequently, peroxynitrite can increase the formation of PGH$_2$ from arachidonate (AA) by stimulating cyclooxygenase while decreasing the formation of prostacyclin by inhibiting prostacyclin synthase (PGIS). Peroxynitrite can also increase the phosphorylation and activation of JNK.

Peroxynitrite is particularly effective with inactivated tyrosine kinases such as CD45. PXCD45 is a JAK phosphatase that appears to have a central role in downregulating proinflammatory responses. The binding site for the phosphate on phosphotyrosine can easily accommodate peroxynitrite and orient it to attack the crucial sulfhydryl in the active site. Cultured endothelium responds to increased shear stress by activating NADPH oxidase and endothelial nitric oxide synthesis. The resulting endogenous formation of peroxynitrite activates c-Jun-NH$_2$-terminal kinase (JNK), which induces a wide range of stress-related responses. The peroxynitrite formation in endothelium does not induce apoptosis. However, peroxynitrite-induced activation of JNK does induce apoptosis in neurons. Peroxynitrite is acting far upstream to activate apoptosis in these studies, and activation of the antiapoptotic serine/threonine kinase Akt (protein kinase B) is sufficient to block cell death. Peroxynitrite may also be promoting intracellular release of acidic fibroblast growth factor, which can induce vascular remodeling and fibroblast proliferation.

While peroxynitrite is acting like a signaling molecule and acting surprisingly far upstream, it is simultaneously oxidizing multiple targets within a cell. DNA, RNA, proteins, and lipids will suffer some extent of oxidative damage at the same time as peroxynitrite is affecting signaling pathways. Why would nature make use of such a damaging agent?

Peroxynitrite and other oxidants are well known to be important microbicidal agents produced by phagocytes. However, peroxynitrite may also be an important adaptive mechanism to limit trauma-induced bleeding and the spread of infective agents. Nitric oxide is almost continuously being produced to modulate vasoconstriction and to reduce platelet adhesion. By activating the production of superoxide, nitric oxide can simultaneously be rapidly inactivated and turned into a potent antimicrobial agent. By having functional groups that are particularly sensitive to peroxynitrite, certain proteins can sense peroxynitrite and thereby reinforce proinflammatory cascades. Resistance to infection is an overwhelming survival factor that is strongly favored by evolution. The collateral damage may be a small price to pay for surviving infection from an evolutionary perspective but may be complicating our current attempts at therapeutic intervention with inhaled nitric oxide.

References

7. Beckman JS, Beckman TW, Chen J, Marshall PM, Freeman BA. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury by nitric oxide and superoxide. Proc Natl Acad Sci U S A. 1990;87:1620–1624.


Key Words: prostacyclin ■ pulmonary hypertension ■ nitrotyrosine ■ superoxide
OONO: Rebounding From Nitric Oxide
Joseph S. Beckman

Circ Res. 2001;89:295-297
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
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:
http://circres.ahajournals.org/content/89/4/295

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/