Rebounding From Nitric Oxide

Joseph S. Beckman

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 al1 have shown that endothelin, the most potent vasoconstrictor known, may contribute to rebound hypertension by inducing superoxide synthase 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 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 peroxynitrite. 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 peroxynitrite 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 Peroxynitrite 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 peroxynitrite is thermodynamically a potent oxidant, it tends to react rather selectively with many biological molecules. The surprising result is that peroxynitrite can activate a variety of signaling pathways, which tend to be proinflammatory and hypertensive (see Figure).

Peroxynitrite promotes the inflammatory synthesis of prostaglandin. The conjugate acid of peroxynitrite (ONO0H) 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 peroxynitrite 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 peroxynitrite, 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.

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

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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 PGH₂ 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 and other oxidants are well known to induce apoptosis in neurons. Peroxynitrite is 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 microbialidal agents produced by phagocytes. However, peroxynitrite may also be an important adaptive mechanism to limit trauma-induced bleeding and the spread of infectious 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.

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