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Forum Editorial

Direct and Indirect Antioxidant Effects of Nitric Oxide: Radically Unsettled Issues

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IT HAS BECOME a textbook erudition that nitric oxide (NO) acts as an inter- and intracellular messenger, and it is implicated in many different biological functions. Diverse and potent effects that NO exerts in normal physiological processes as well as in different diseases are due mainly to its widespread distribution in tissues and to its ability to bind and interact with hemoproteins such as soluble guanylyl cyclase, hemoglobin, myoglobin, and peroxidases. These functions of NO are not directly associated with its specific propensity to act as a free radical. In contrast, a recent discovery of a direct free radical scavenging role of NO is due solely to its free radical nature (reviewed in 9).

The overwhelming majority of structural and functional biomolecules in cells and biological fluids are not free radicals. Therefore, a reactive free radical once formed will most likely interact with these nonradical molecules to produce new radicals. As a result, free radical processes have a tendency to propagate as chain reactions and spread around to involve more and more new neighboring molecules, thus causing substantial damage. Clearly, recombination of two radicals might be an effective way to terminate a free radical process. Unfortunately, the collision of a free radical with another reactive free radical is not that likely. Being a free radical, NO offers a unique opportunity to scavenge reactive propagating free radicals and terminate damaging free radical processes.

This seemingly simple and unequivocally advantageous function of NO is, however, as controversial as its other roles. Even aside from its calamitously known interaction with superoxide radical resulting in the production of peroxynitrite (reviewed in 1, 13), the products of NO recombination with different radicals may be unstable. For example, interactions of NO with lipid peroxyl radicals ($LOO^{\cdot}$) result in a very effective inhibition of lipid peroxidation (reviewed in 9). NO turns out to be more effective as a radical scavenger than vitamin E, and combination of the latter with NO was found to be more effective than vitamin E/vitamin C couple (24). However, molecular products formed during the reaction of NO with peroxyl radicals, organic peroxynitrites, are not particularly stable and may be decomposed to generate the more reactive alkoxyl and nitrogen dioxide free radicals, or the less reactive organic nitrate (17). Obviously, the gain that is achieved through this type of elimination of peroxyl radicals by interaction with NO may be weakened by the new radicals formed. Although NO has been recognized as an important scavenger of lipid-derived radicals, its role in antioxidant protection against thiyl radicals ($RS^{\cdot}$), although predictable, has not been well documented experimentally (4).

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Further studies are necessary to provide strong evidence that accumulation of nitrosothiols (*e.g.*, in sepsis and preeclampsia) is due to scavenging of thiyl radicals by NO.

Effective binding of NO with hemoproteins and non-heme iron catalytic sites predisposes its chemical interactions with iron-catalyzed reactions. In particular, NO can act as a direct chemical reductant during its interactions with potent oxidants formed in the presence of H_2O_2 or organic peroxides, oxoferryl species. This reaction has been suggested as an important antioxidant mechanism against iron-mediated oxidative stress (5, 8, 14). The same mechanisms, however, can be operational toward catalase- and peroxidase-catalyzed reactions in which case their prooxidant rather antioxidant role seems more apparent (10, 11).

Another important pathway through which NO can affect overall redox balance is its ability to nitrosylate (oxidize) protein thiolate clusters involved in binding of metals, including transition metals. As a result, bound metals can be "loosened" or released from proteins. In the case of transition metals, their fate is crucial for determining subsequent pro- or antioxidant events. For example, copper bound by metallothioneins and released by NO may be utilized to reconstitute apo-Zn-superoxide dismutase (apo-Zn-SOD) to functional Cu,Zn-SOD yielding enhanced antioxidant protection. If reconstitution of SOD activity is not the predominant pathway for released copper, its presence in redox-active form may cause enhanced redox-cycling activity and oxidative damage to cells.

Finally, a plethora of antioxidant and prooxidant effects of NO may arise as a result of its actions in regulating expression of proteins involved in prooxidant metabolism. For example, NO regulates the "iron response element binding protein" (IRE-BP), an important mRNA-binding protein, that is a key regulatory element for the synthesis of proteins involved in iron metabolism (7, 22, 27). IRE-BP binds to the mRNA of genes such as the transferrin receptor, ferritin, and erythroid aminolevulinic acid synthase, and controls the translation and/or stability of the messages (6, 20, 27, 29). An important iron-sulfur cluster containing IRE-BP and target for NO is cytoplasmic aconitase. By reacting with this protein, NO modulates its mRNA binding functions as well as its enzyme activity. This, in turn, can modulate cellular iron homeostasis and production of reactive oxygen intermediates, as well as redox status. The effects of NO will depend on the cell type, availability of iron, and localized concentrations of H_2O_2 (21, 27).

Altering expression of proteins conferring resistance to free radical damage remains a key element defining the pathways by which NO can act as an indirect antioxidant. One important protein mediating this function is heme oxygenase-1, the rate-limiting enzyme in heme catabolism. In many different cell types, NO induces heme oxygenase-1 (2, 11), and several mechanisms have been suggested to explain the role of this protein in defense against oxidant injury (15, 19, 28). Importantly, heme is considered to be a dangerous intracellular prooxidant, and its breakdown by heme oxygenase-1 is an important protective mechanism (16). Heme oxygenase-1 activity generates bilirubin, carbon monoxide, and iron. Carbon monoxide and bilirubin have antioxidant potential, carbon monoxide has been reported to protect against hyperoxic lung injury (16), and bilirubin can act as a peroxyl radical scavenger (25, 26). Although iron can generate free radicals via Fenton-type chemistry, it also induces ferritin, which can sequester this metal and effectively limit its toxicity (16). Examples of several additional genes important in redox regulation in cells regulated by NO include the heme-containing proteins catalase and cytochrome P450 (3, 10, 11), heat-shock protein 70 (13), and hypoxia-inducible factor-1 (12, 18).

Overall, NO is not an indisputable antioxidant. It may affect numerous metabolic pathways acting directly or indirectly in an anti- and/or prooxidant fashion. The final balance in favor of its anti- or prooxidant role(s) is dependent on specific predominant mechanism(s) of oxidative stress in each cell. Identification of these antioxidant or prooxidant functions of NO will be a challenge for future studies in the field of NO research.

ABBREVIATIONS

IRE-BP, iron response element binding protein; NO, nitric oxide; SOD, superoxide dismutase.

REFERENCES

1. Arteel GE, Briviba K, and Sies H. Protection against peroxynitrite. *FEBS Lett* 445: 226–230, 1999.
2. Bouton C, and Demple B. Nitric oxide-inducible expression of heme oxygenase-1 in human cells. Translation-independent stabilization of the mRNA and evidence for direct action of nitric oxide. *J Biol Chem* 275: 32688–32693, 2000.
3. Calabrese, V, Copani A, Testa D, Ravagna A, Spadaro F, Tendi E, Nicoletti VG, and Giuffrida Stella AM. Nitric oxide synthase induction in astroglial cell cultures: effect on heat shock protein 70 synthesis and oxidant/antioxidant balance. *J Neurosci Res* 60: 613–622, 2000.
4. Chiueh CC. Neuroprotective properties of nitric oxide. *Ann N Y Acad Sci* 890: 301–311, 1999.
5. Cooper CE. Nitric oxide and iron proteins. *Biochim Biophys Acta* 1411: 290–309, 1999.
6. Drapier JC, Hirling H, Wietzerbin J, Kaldy P, and Kuhn LC. Biosynthesis of nitric oxide activates iron regulatory factor in macrophages. *EMBO J* 12: 3643–3649, 1993.
7. Gonzalez-Flecha B, and Demple B. Genetic responses to free radicals. Homeostasis and gene control. *Ann N Y Acad Sci* 899: 69–87, 2000.
8. Gorbunov NV, Yalowich JC, Gaddam A, Thampatty P, Ritov VB, Kisin ER, Elsayed NM, and Kagan VE. Nitric oxide prevents oxidative damage produced by *tert*-butyl hydroperoxide in erythroleukemia cells via nitrosylation of heme and non-heme iron. Electron paramagnetic resonance evidence. *J Biol Chem.* 272: 12328–12341, 1997.
9. Hogg N, and Kalyanaraman B. Nitric oxide and lipid peroxidation. *Biochim Biophys Acta* 1411: 378–384, 1999.
10. Kim YM, Bergonia HA, Muller C, Pitt BR, Watkins WD, and Lancaster JR Jr. Nitric oxide and intracellular heme. *Adv Pharmacol* 34: 277–291, 1995.
11. Kim YM, Bergonia HA, Muller C, Pitt BR, Watkins WD, Lancaster JR Jr. Loss and degradation of enzyme-bound heme induced by cellular nitric oxide synthesis. *J Biol Chem* 270: 5710–5713, 1995.
12. Kimura H, Weisz A, Ogura T, Hitomi Y, Kurashima Y, Hashimoto K, D'Acquisto F, Makuuchi M, and Esumi H. Identification of hypoxia-inducible factor-1 (HIF-1) ancillary sequence and its function in vascular endothelial growth factor gene induction by hypoxia and nitric oxide. *J Biol Chem* 276: 2292–2298, 2001.
13. Koppenol WH. Chemistry of peroxynitrite and its relevance to biological systems. *Met Ions Biol Syst* 36: 597–619, 1999.
14. Liochev SI, and Fridovich I. Superoxide and iron: partners in crime. *IUBMB Life* 48: 157–161, 1999.
15. Otterbein L, Sylvester SL, and Choi AM. Hemoglobin provides protection against lethal endotoxemia in rats: the role of heme oxygenase-1. *Am J Respir Cell Mol Biol* 13: 595–601, 1995.
16. Otterbein LE, and Choi AM. Heme oxygenase: colors of defense against cellular stress. *Am J Physiol Lung Cell Mol Physiol* 279: L1029–L1037, 2000.
17. Padmaja S, and Huie RE. The reaction of nitric oxide with organic peroxyl radicals. *Biochem Biophys Res Commun* 195: 539–544, 1993.
18. Palmer LA, Gaston B, and Johns RA. Normoxic stabilization of hypoxia-inducible factor-1 expression and activity: redox-dependent effect of nitrogen oxides. *Mol Pharmacol* 58: 1197–1203, 2000.
19. Panahian N, Yoshiura M, and Maines MD. Overexpression of heme oxygenase-1 is neuroprotective in a model of permanent middle cerebral artery occlusion in transgenic mice. *J Neurochem* 72: 1187–1203, 1999.
20. Pantopoulos K, and Hentze MW. Nitric oxide signaling to iron-regulatory protein: direct control of ferritin mRNA translation and transferrin receptor mRNA stability in transfected fibroblasts. *Proc Natl Acad Sci U S A* 92: 1267–1271, 1995.
21. Pantopoulos K, Weiss G, and Hentze MW. Nitric oxide and oxidative stress (H_2O_2) control mammalian iron metabolism by different pathways. *Mol Cell Biol* 16: 3781–3788, 1996.
22. Ponka P. Cell biology of heme. *Am J Med Sci* 318: 241–256, 1999.
23. Rubbo H, Radi R, Trujillo M, Telleri R, Kalyanaraman B, Barnes S, Kirk M, and Freeman BA. Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation. Formation of novel nitrogen-containing oxidized lipid derivatives. *J Biol Chem* 269: 26066–26075, 1994.
24. Rubbo H, Radi R, Anselmi D, Kirk M, Barnes S, Butler J, Eiserich JP, and Freeman BA. Nitric oxide reaction with lipid peroxyl radicals spares alpha-tocopherol during lipid peroxidation. Greater oxidant protection from the pair nitric oxide/alpha-tocopherol than alpha-tocopherol/ascorbate. *J Biol Chem* 275: 10812–10818, 2000.
25. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, and Ames BN. Bilirubin is an antioxidant of possible physiological importance. *Science* 235: 1043–1046, 1987.
26. Stocker R, Glazer AN, and Ames BN. Antioxidant activity of albumin-bound bilirubin. *Proc Natl Acad Sci U S A* 84: 5918–5922, 1987.
27. Theil EC. Targeting mRNA to regulate iron and oxygen metabolism. *Biochem Pharmacol* 59: 87–93, 2000.
28. Vile GF, Basu-Modak S, Waltner C, and Tyrrell RM. Heme oxygenase 1 mediates an adaptive response to oxidative stress in human skin fibroblasts. *Proc Natl Acad Sci U S A* 91: 2607–2610, 1994.
29. Weiss G, Goossen B, Doppler W, Fuchs D, Pantopoulos K, Werner-Felmayer G, Wachter H, and Hentze MW. Translational regulation via iron-responsive elements by the nitric oxide/NO-synthase pathway. *EMBO J* 12: 3651–3657, 1993.

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1. Marcela A Avila, Stacy L Sell, Yuji Kadoi, Donald S Prough, Helen L Hellmich, Marco Velasco, Douglas S Dewitt. 2008. L-Arginine decreases fluid-percussion injury-induced neuronal nitrotyrosine immunoreactivity in rats. *Journal of Cerebral Blood Flow & Metabolism* **28**:10, 1733-1741. [CrossRef]
2. Hülya Bay#r, Valerian E Kagan, Grigory G Borisenko, Yulia Y Tyurina, Keri L Janesko, Vincent A Vagni, Timothy R Billiar, Deborah L Williams, Patrick M Kochanek. 2005. Enhanced oxidative stress in iNOS-deficient mice after traumatic brain injury: support for a neuroprotective role of iNOS. *Journal of Cerebral Blood Flow & Metabolism* **25**:6, 673-684. [CrossRef]
3. Ted H. Elsasser, Stas Kahl. 2002. Adrenomedullin has multiple roles in disease stress: Development and remission of the inflammatory response. *Microscopy Research and Technique* **57**:2, 120-129. [CrossRef]