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Forum Review

Mechanisms of the Antioxidant Effects of Nitric Oxide

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ABSTRACT

The Janus face of nitric oxide (NO) has prompted a debate as to whether NO plays a deleterious or protective role in tissue injury. There are a number of reactive nitrogen oxide species, such as N_2O_3 and $ONOO^-$, that can alter critical cellular components under high local concentrations of NO. However, NO can also abate the oxidation chemistry mediated by reactive oxygen species such as H_2O_2 and O_2^- that occurs at physiological levels of NO. In addition to the antioxidant chemistry, NO protects against cell death mediated by H_2O_2, alkylhydroperoxides, and xanthine oxidase. The attenuation of metal/peroxide oxidative chemistry, as well as lipid peroxidation, appears to be the major chemical mechanisms by which NO may limit oxidative injury to mammalian cells. In addition to these chemical and biochemical properties, NO can modulate cellular and physiological processes to limit oxidative injury, limiting processes such as leukocyte adhesion. This review will address these aspects of the chemical biology of this multifaceted free radical and explore the beneficial effect of NO against oxidative stress. Antioxid. Redox Signal. 3, 203–213.

INTRODUCTION

ENDOGENOUS FORMATION of the radical species nitric oxide (NO) results in an array of biological effects. Over the last decade, the role that NO plays in oxidative injury has been debated (2, 8, 23, 55). In the presence of oxygen (O_2) or reactive oxygen species (ROS) such as superoxide (O_2^-), NO is converted into reactive nitrogen oxide species (RNOS), which can irreversibly modify a variety of biological molecules (summarized in 54). Thus, NO is often considered to be a toxic species. However, NO has also been shown to abate oxidative injury in several biological systems. This review includes a discussion of the diverse chemistry of NO, which can result in processes as disparate as abatement of oxidative stress and mediation of cell death and tissue injury. Also discussed are the mechanisms by which NO modifies cellular processes that protect cells and tissue from oxidative damage. Finally, the effects of NO treatment in various disease models are reviewed.

One of the fundamental determinants of the role of NO *in vivo* is its chemistry. As NO is a radical, it has a number of potential chemical pathways in biological systems. As discussed in previous articles, the complex chemistry of NO can be divided primarily into the two cat-

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egories of direct and indirect effects (54). Direct effects are comprised of those reactions in which NO interacts directly with the biological target. Conversely, indirect effects are mediated by RNOS formed from the reaction of NO with O_2 or O_2^-.

One advantage to categorizing the reactions of NO in this manner is that the concentration of NO can be used to predict the contribution of each effect. Direct effects require low concentrations of NO, whereas indirect effects occur at much higher NO concentrations (54). Toxicity largely occurs under conditions where NO-derived species are formed via the indirect route. Thus, deleterious effects derived from the chemistry of NO are often confined to conditions of high local NO concentrations. The antioxidant effects of NO are primarily a result of direct effects at lower NO concentrations.

CHEMISTRY OF THE ANTIOXIDANT PROPERTIES OF NO

An antioxidant is a substance that prevents oxidant formation or scavenges oxidants produced under conditions of oxidative stress. The primary source of oxidative stress is ROS, such as O_2^- and peroxide. These species often produce oxidants through Fenton-type reactions. RNOS, such as peroxynitrite ($ONOO^-$), nitrogen dioxide (NO_2), and nitroxyl (HNO), are another potential source of oxidative stress, although they are less potent oxidants than those formed as a result of Fenton-type reactions. Further, lipid peroxidation perpetuates oxidative stress through formation of a variety of lipid-oxy and -peroxy adducts. The versatile chemistry of NO provides antioxidant mechanisms against all three of these types of oxidants predominantly through radical–radical and ligand–metal interactions.

Fenton chemistry

In general, Fenton-type reactions occur between peroxide and transition metals and result in formation of hydroxyl radicals or hypervalent peroxo- or oxo-metal complexes.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH, Fe^{4+}O, FeO_2 \quad (1)$$

These reactive intermediates can then alter biological substances such as proteins, nucleic acids, and lipids, resulting in tissue injury (54). NO can abate Fenton-mediated oxidative stress by direct scavenging of oxidants, prevention of peroxide reaction, and scavenging of reducing equivalents supplied by O_2^- (Fig. 1).

Hydroxyl radicals and high valent metal complexes are scavenged by NO at near diffusion control (*e.g.*, $>10^9\ M^{-1}\ s^{-1}$ for NO + ${}^{\bullet}OH$; 4). However, reactions between these highly reactive compounds and substances other than NO can also proceed with similarly high second-order rate constants (21, 22). Therefore, the determining factor for the antioxidant properties of NO is the relative concentration of NO with respect to other potential reaction sites (*i.e.*, the pseudo first-order rate constants). As discussed below, lipid peroxidation is abated through a radical–radical reaction mechanism at biologically relevant concentrations of NO (48). Conversely, the reaction products of the Fenton reaction generally must be produced in close proximity to a macromolecule to induce DNA strand breaks or protein oxidation. Therefore, diffusion of NO to these regions would likely not yield high enough effective NO concentrations to out-compete the deleterious reactions between oxidants and biomolecules. Under these conditions, NO would not

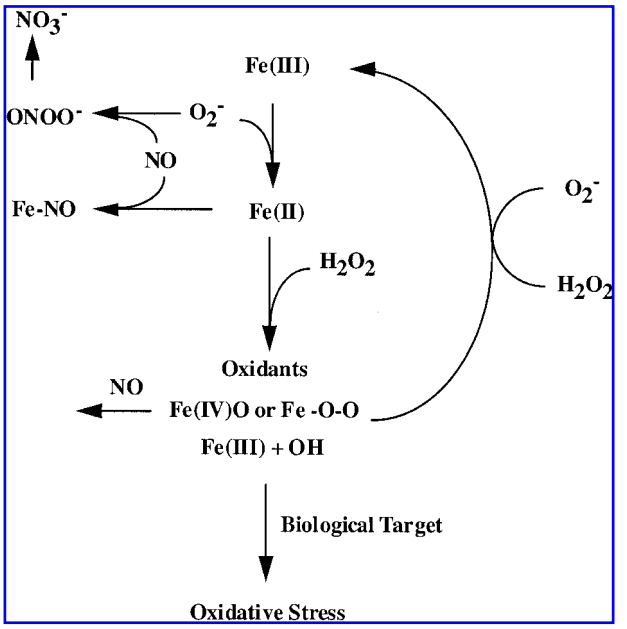

FIG. 1. Antioxidant mechanism of NO in the Fenton-type reactions.

be a good scavenger of oxidants and probably would not have an antioxidant role.

The antioxidant properties of NO also include prevention of oxidant formation. For instance, formation of a metal nitrosyl complex can prevent addition of peroxide to the metal coordination site. Metals such as copper and nickel have been invoked as mediators of oxidative stress in some cases, however, iron complexes are the predominant catalyst for this reaction *in vivo*, generally through Fenton-type reactions. To understand better the relationship between iron nitrosyl complex formation and prevention of oxidant production, it is useful to consider the environment of the interacting metal and ligands.

Hard–soft acid–base rules state that hard ligands, which donate their electrons less readily than soft ligands, tend to associate more strongly with hard, low polarizable metals. For instance, ferric iron, which is a hard metal, will bind more tightly to the hard ligand hydroxide than will the softer ferrous ion. However, binding of soft ligands can result in softening of hard metals. The ability of ligands to alter the metal environment affects the reactivity of metal-catalyzed reactions, which depend on the oxidation state of the metal as well as the ligand field.

For instance, hydroxyl radical is the primary product of the reaction between peroxide and soft ferrous iron surrounded by a hard ligand field. In softer ligand fields, such as provided by heme complexes, both the ferric and ferrous states principally produce oxidizing species such as Fe^{4+} and Fe^{5+} oxo complexes rather than hydroxyl radicals. As NO is a soft ligand, it will readily bind to ferrous complexes to form a metal-nitrosyl species, but as a general rule, if peroxide can react with the metal site, NO can also bind. The resulting nitrosyl complex inhibits the reaction between peroxide and the metal, thereby preventing ROS production.

As described above, formation of hydroxyl radicals or metal-oxo species often requires Fe^{2+}. However, iron is more stable in the ferric valence state *in vivo*. Reduction of Fe^{3+} to Fe^{2+} (Haber–Weiss chemistry) can involve O_2^-, which can provide the electrons to facilitate catalytic oxidation of biological compounds (20). NO and O_2^- react at diffusion control to form $ONOO^-$, which rapidly rearranges to nitrate in the absence of other reactive species (45). Shunting of O_2^- to nitrate thus inhibits reduction of ferric iron and prevents catalytic formation of ROS. This may be an important mechanism for abatement of Fenton-type reaction-mediated oxidative stress by NO.

NO/O_2^- reaction

Although scavenging of O_2^- by NO can prevent production of ROS via the Fenton reaction, $ONOO^-$ is itself an oxidant. Exposure to synthetic $ONOO^-$ has been shown to induce tissue injury through reactions such as DNA damage and lipid peroxidation (46). Therefore, formation of $ONOO^-$ from the reaction between O_2^- and NO has been speculated to induce oxidative stress *in vivo* (2, 46). However, the chemistry of the NO/O_2^- reaction can often give different results from exposure to synthetically generated $ONOO^-$.

The oxidative chemistry of the NO/O_2^- reaction was found to depend on the relative rate of production of the two radicals (41, 59). For instance, in the presence of a constant O_2^- flux from xanthine oxide/hypoxanthine (XO), oxidation of dihydrorhodamine or glutathione (GSH) increased with increasing NO until the rates of formation of these radicals were equivalent. Further increases in NO concentration, produced from a class of compounds known as NONOates, which release NO in a controlled manner over specific time periods (31), resulted in marked decreases in dihydrorhodamine or GSH oxidation. These data demonstrate that, in the presence of excess NO, the reactive intermediates formed during decomposition of $ONOO^-$ are scavenged by NO. This reaction ultimately results in production of the nitrosating species N_2O_3 (Fig. 2; 59). These findings indicate that the oxidative chemistry of the NO/O_2^- reaction is confined to a small range of fluxes because excess NO can convert oxidative RNOS to nitrosating species.

More recently, carbon dioxide (CO_2) was shown to react with $ONOO^-$ to form a potent adduct, CO_2OONO^-, which has oxidative properties similar to those of $ONOO^-$ (17, 36, 38). However, CO_2 activation of $ONOO^-$ is pH-independent, unlike formation of the reactive species HOONO in the absence of CO_2. Excess NO also quenches the oxidation chemistry of this

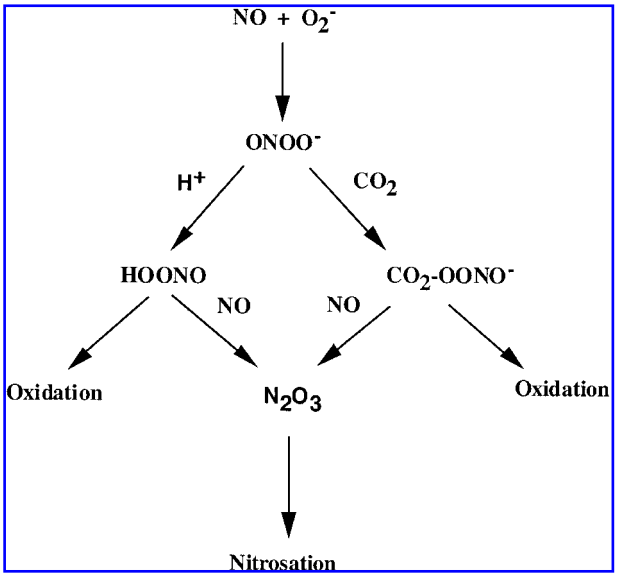

FIG. 2. Chemistry of reaction between NO and O_2^-.

adduct (28). The CO_2 adduct has been proposed to decompose to the carbonate radical (CO_3^-) and NO_2 (38). Reaction of excess NO with NO_2 formed in this manner would again result in production of N_2O_3 (Fig. 2; 18, 28, 54). These studies suggest that the spatial positioning as well as timing of formation of these two radicals will determine the extent of RNOS-mediated oxidation and that oxidative stress and nitrosative stress are balanced within the NO/O_2^- reaction. Therefore, nitrosative stress may provide an optimal antioxidant environment.

Lipid peroxidation

The process of lipid peroxidation results in formation of a variety of lipid-oxy and -peroxy adducts (21). Perpetuation of lipid oxidation by these species can result in cell membrane compromise (Fig. 3). Reaction of NO with these peroxy and oxy radicals terminates lipid peroxidation via Eq. 2 and results in protection against ROS (45):

$$LOO^{\bullet} + NO \rightarrow LOONO \qquad (2)$$

where $LOO^{\bullet}$ is the lipid peroxy radical. Chain termination also prevents oxidation of low density lipoproteins in both endothelial cells (52) and macrophages (26). Reduction in oxidized cholesterol is thought to reduce initiation of atherosclerosis mediated by foaming macrophages. Other inflammatory processes such as production of leukotrienes are also effected by NO (54, and references therein). Lipoxygenase, which mediates a variety of lipid oxidation, is inhibited by NO (54, and references therein). Thus, termination of lipid peroxidation may be one of the most important antioxidant properties of NO.

CHEMICAL TOXICOLOGY OF NO AND ROS

Although NO often acts as an antioxidant, the chemistry of NO can also affect cellular processes such that cells or tissue becomes more susceptible to oxidative stress. This complexity of potential NO reactions within the cell has led to differing opinions on the role of NO in oxidative stress (23). The effect of NO on the toxicity of hydrogen peroxide (H_2O_2), alkylhydroperoxide, and O_2^- at the cellular level was therefore examined by clonogenic assay, which is the primary means for determining the cytotoxicity of chemical substances as it accounts for both necrotic and apoptotic death.

Cytotoxic effects of NO/H_2O_2

H_2O_2 mediates oxidation of biological molecules, which can result in tissue damage. Although NO does *not* react directly with H_2O_2 (55), it can protect cells against H_2O_2-mediated toxicity (19, 55, 56, 57). Exposure of lung fibro-

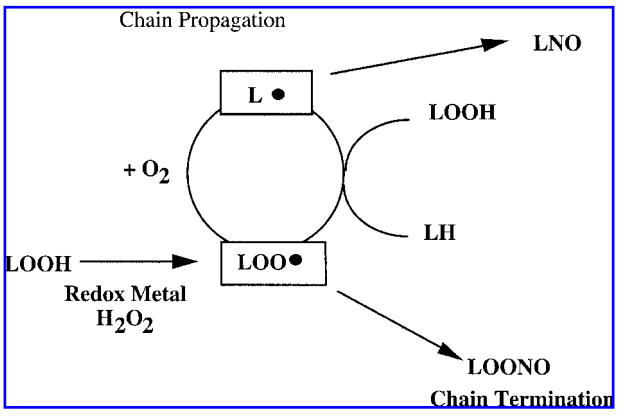

FIG. 3. Mechanism for termination of lipid peroxidation by NO.

blasts to increasing concentrations of H_2O_2 induced marked increases in cytotoxicity (55). Addition of NONOate compounds surprisingly resulted in protection against the cytotoxicity of H_2O_2 (55). Pre- or posttreatment with these NO donor complexes did not result in protection; in fact, the by-product of the decomposition of NO, nitrite, increased the cytotoxicity of H_2O_2. Similar observations were made in neuronal (55), hepatoma (56), and endothelial cells (6, 19).

These protective effects of NO were not restricted to NONOates, as endogenous formation of NO in endothelial cells was also suggested to be involved in protection against damage to vascular smooth muscle mediated by H_2O_2 (37). Further, the *S*-nitroso-containing compounds *S*-nitrosothiolglutathione (GSNO) and *S*-nitroso-*N*-acetylpenicillamine (SNAP) also protected against H_2O_2-mediated toxicity (58). However, nitrovasodilators commonly used in the clinic, such as 3-morpholinosydnonimine (SIN-1) and sodium nitroprusside (SNP), increased the toxicity of H_2O_2 (14, 58). Angeli's salt (AS; $Na_2N_2O_3$), which is structurally similar to the NONOate compounds but donates nitroxyl (NO^-) instead of NO, significantly potentiated the toxicity of H_2O_2 (58). These results demonstrate that the common putative NO donors modulate the toxicity of H_2O_2 differently, and so caution is required in the interpretation of experimental results.

The diverse effects exhibited by the NO donors examined may be explained by variance in donor effect on cellular antioxidant defenses and in amount and flux of NO produced. One of the major cellular defenses against H_2O_2 is consumption of this oxidant by the enzymes glutathione peroxidase and catalase (21, 22). When the kinetics for the disappearance of H_2O_2 were examined in the presence of the different NO donors, cellular consumption of H_2O_2 was noted to be inhibited to varying degrees by several of the donors. For instance, the amount of time required to decompose 0.75 m*M* H_2O_2 in the presence of SNP, the NONOate $Et_2NN(O)NO$ (DEA/NO), AS, and SNAP increased by 30–200% (58). Conversely, SIN-1 and GSNO retarded H_2O_2 consumption by as much as 400%. Thus, enhancement of H_2O_2-mediated toxicity by AS and SIN-1 might be partially explained by inhibition of H_2O_2 consumption. However, this cannot be the sole mechanism by which NO enhances or protects against H_2O_2, because GSNO, SNAP, and DEA/NO also decreased the rate of decomposition of H_2O_2, but proved to be cytoprotective.

Furthermore, cellular exposure to the NO donors resulted in varied reduction of intracellular levels of GSH. Exposure of V79 cells to 1 m*M* nitrite, SNAP, SIN-1, GSNO, DEA/NO, or AS for 1 h resulted in varying degrees of depletion of intracellular GSH (60). For instance, SNAP, GSNO, and DEA/NO resulted in only modest GSH decreases (<30%), which were recovered rapidly. Conversely, SIN-1 and AS decreased intracellular GSH levels by as much as 85%, whereas nitrite decreased GSH levels by 50% in these cells. This reduction in cellular GSH may be the mechanism by which SIN-1, AS, and nitrite enhance H_2O_2 toxicity.

The observed differences in protective effects among the various chemical NO donors may also be a reflection of the actual flux of NO produced by each compound. The temporal profiles of NO release by the different compounds, as assessed with an NO-selective electrode, demonstrate that the amount of NO released over time is quite variant (58). Both the NONOates and *S*-nitroso complexes, which protected against H_2O_2 toxicity, released NO over the entire time course of exposure to H_2O_2 (1 h). However, SIN-1, SNP, and AS did not produce measurable NO (<1 μM) under these experimental conditions, coincident with a lack of protection against H_2O_2.

SNP appears to increase the toxicity of ROS by yet another mechanism. Chemical interaction with SNP can result in formation of not only NO, but also cyanide (CN^-) and free iron. The iron chelator desferrioxamine (DF) completely protected cells from H_2O_2, yet only partially protected against the toxicity of H_2O_2 combined with SNP (58). This discrepancy may be accounted for by enhanced release of CN^- from SNP. Monocytes and polymorphonuclear leukocytes have also been shown to facilitate release of CN^- from SNP, which is a phenomenon believed to be mediated by H_2O_2 (5). A transition metal complex with a labile ligand was suggested to oxidize substrates further via Fenton-type catalysis (58). Further evidence supporting

this hypothesis comes from Imlay and colleagues, who showed that bacteria became more sensitive to H_2O_2 in the presence of CN^- (27). The fact that DF completely protected against the toxicity of CN^- suggests that metal–peroxide reactions are required to initiate cytotoxicity. Thus, the DF-insensitive enhancement of H_2O_2-mediated toxicity by SNP could be attributed to an iron complex, which cannot be bound by DF, and so could catalyze the Fenton oxidation chemistry of cellular molecules.

Cytotoxic effects of NO/alkylperoxides

NO was also found to act as an antioxidant against XO-mediated lipid peroxidation (49). We examined the effect of NO on organic hydroperoxide-mediated toxicity, which is thought to be mediated by oxidation of lipophilic membranes (57). Our studies further illustrate the importance NO production for the duration of exposure to oxidants. DEA/NO, which is a NONOate with a half-life of ~2 min at 37°C, provided minimal protection against either *tert*-butyl hydroperoxide or cumene hydroperoxide, whereas the longer lived PAPA/NO (half-life of 15 min at 37°C) exhibited marked protection. The different effects of these two NONOates on cytotoxicity can be attributed to the timing of delivery of NO as exposure to organic peroxides usually requires up to 2 h to induce observable toxicity (57). As alkylhydroperoxides require longer times to penetrate cells and thereby exert their damage, longer sustained fluxes of NO are more protective.

Several potential mechanisms may be involved in the protection by NO against organic hydroperoxide-mediated toxicity. Intracellular metalloproteins, such as those containing heme moieties, react quickly with organic peroxides to form hypervalent complexes (22). Upon decomposition, these complexes release intracellular iron, which in turn can catalyze damage to macromolecules such as DNA. NO can react with these hypervalent metalloproteins at near diffusion control, which may restore these oxidized species to the ferric form (16, 50). Reduction of these metal-oxo proteins prevents both their oxidative chemistry and their release of free iron (16, 29, 56), thus limiting intracellular damage mediated by oxidative stress.

Interestingly, although NO can protect against the toxicity of H_2O_2 in mammalian cells, the opposite effect is observed in *E. coli*. Delivery of H_2O_2 either as a bolus or through the enzymatic activity of XO, exhibited only modest bactericidal activity (44). However, simultaneous exposure to both H_2O_2 and NO, delivered either as gas or by a NONOate complex, increased bactericidal activity by four orders of magnitude. Addition of catalase abated toxicity, whereas exposure to superoxide dismutase (SOD) had no effect. These results demonstrate that the synergistic action of NO and H_2O_2 rather than O_2^- was responsible for this bactericidal activity. Thus, the combination of NO and H_2O_2 may be ideally suited to combat *E. coli* infections due to the additional protective effect of NO for the host. This mechanism may also apply to other species of bacteria, albeit with different kinetics. For instance, the cytotoxicity of O_2^- in *Staphylococcus* was abrogated by NO at early time points, yet NO helped sustain toxicity at longer time intervals. The maximal effect was dependent upon the timing of exposure to NO, H_2O_2 and O_2^- (30). These findings may explain why NO and ROS are produced by immune effector cells at different times following exposure to different pathogens.

The diametric responses of mammalian cells and prokaryotes to the combination of NO/H_2O_2 may reflect their different cellular structures and complement of metalloproteins. Bacteria utilize iron-sulfur clusters to a greater extent than do mammalian cells, and these types of proteins are especially susceptible to degradation mediated by NO or RNOS (12, 24). In *E. coli*, decomposition of iron complexes occurs in the periplasmic space, which is in close proximity to the cytoplasm. This relative lack of compartmentalization may allow iron to bind to and oxidize DNA. However, due to the organelle structure of mammalian cells, metal labilization may be limited to the cytoplasm and mitochondria. In such a cellular arrangement, metals would be required to travel a large distance to reach the nucleus and bind to DNA.

Cytotoxic effects of NO/O_2^-

Treatment of cells with $ONOO^-$ results in cell death in both bacterial (62) and mammalian

systems (reviewed in 46). However, treatment of lung fibroblasts and neurons with O_2^- and NO did not exhibit appreciable toxicity (55). Other studies showed that ovarian carcinoma cells exposed to 5 m*M* SIN-1, which is a simultaneous generator of NO/O_2^-, did not result in appreciable toxicity (14). In fact, cells treated concomitantly with O_2^- and NO releasing compounds resulted in protection against O_2^--mediated toxicity and *did not* display appreciable toxicity due to $ONOO^-$ formation (58). These results suggest that there is a distinct difference between treating cells with bolus concentrations of synthetic $ONOO^-$ (millimolar) and generating $ONOO^-$ with NO/O_2^- systems.

Part of the discrepancy between bolus administration and *de novo* synthesis of $ONOO^-$ can be explained in terms of reactant concentrations. Beckman and co-workers described that penetration into cells by bolus treatment required high concentrations of $ONOO^-$ because the cell membrane forms a formidable barrier for $ONOO^-$ penetration to intracellular targets (62). Although extracellular generation of NO and O_2^- results in $ONOO^-$ formation, the short lifetime of this species in solution does not allow accumulation of high enough concentrations to penetrate the cell. Therefore, the amount of $ONOO^-$ that could cross the cellular membrane under more biologically relevant conditions than bolus administration, despite production of stoichiometrically high amounts over a prolonged time period, is dramatically reduced. The cell membrane thus limits the contribution of extracellular $ONOO^-$ to toxicological mechanisms.

Another factor to consider with respect to toxicity mediated directly by $ONOO^-$ chemistry is that the reaction between NO and $ONOO^-$ forms NO_2, as was discussed above. Competition for O_2^- by cellular components such as SOD and redox proteins increases the amount of NO required to form $ONOO^-$. As the NO flux then exceeds the O_2^- flux, $ONOO^-$ is converted to potent nitrosating agents. Hence, the chemistry of extracellular formation of $ONOO^-$ by excess NO converts $ONOO^-$ to nitrite. Direct necrotic cell death mediated by oxidative chemistry of $ONOO^-$ from exposure of simultaneous NO/O_2^- derived from NADPH is thus unlikely.

CELLULAR AND PHYSIOLOGICAL EFFECTS OF NO AND OXIDATIVE STRESS

In addition to abating oxidative stress chemically, NO can protect against oxidative stress at the cellular level. For example, pretreatment with NO several hours prior to exposure can confer protection against H_2O_2 (43). In bacteria, treatment with NO resulted in up-regulation of the SOXR and subsequent expression of protective proteins against ROS (43). Hepatocytes also became resistant to peroxide injury following treatment with NO (33). Expression of hemoxygenase as well as other enzymes may participate in NO-induced protection against peroxide (33). In addition to stimulating expression of protective proteins, NO has a number of effects on cellular functions that may influence oxidative stress. For instance, the presence of NO may limit ROS production by preventing assembly of NADPH oxidase, which is one of the major contributors to oxidative stress in various immune responses (9).

In addition to limiting interfering with oxidative stress, NO has been shown to prevent the induction of some genes that are induced from ROS. For instance, oxidative stress can induce early growth response-1, which through the extracellular signal–regulated kinase pathway can activate a number of adhesion molecules. It has been shown that NO derived from a donor or endothelial NO synthase (eNOS) abates the up-regulation of this system (7).

NO can also inhibit oxidative stress processes at physiological levels, which can result in protection against tissue injury. NO has been shown to attenuate ischemia–reperfusion injury in numerous organs such as heart, intestine, liver, kidney, and lung. To illustrate, NO, either from donors or produced endogenously, was shown to protect against myocardial ischemia–reperfusion injury (39, 51). Endogenous NO can also attenuate lung damage after intestinal ischemia. These reports suggest that under different conditions where ischemia–reperfusion is involved NO can abate tissue damage.

Neutrophil and leukocyte adhesion is also inhibited by NO (34, 39). Leukocyte adhesion and extravasation to the endothelium is one of

the primary mechanisms by which tissue damage is mediated in ischemia–reperfusion. Preventing an increase in activated leukocytes in these regions is thought to protect tissue from ischemia–reperfusion injury (34, 39). Increased leukocyte adhesion and infiltration involve a number of proinflammatory molecules, including vascular cell adhesion molecule, intercellular adhesion molecule (ICAM), and CD11/CD18 expression (1, 11, 32, 53), and are induced by ROS formation such as superfused XO in the rat mesentery (15). This response could be inhibited by treatment with either SOD or NO donors, which scavenged O_2^-, thus preventing activation of the cell adhesion molecule, P-selectin. It also has been shown that monocyte chemotactic protein-1 induced by oxidative stress is inhibited by NO derived either from an NO donor or from NOS-3 (53). In summary, oxidative stress-induced leukocyte adhesion mechanisms are inhibited by the presence of NO.

NO also abates oxidative chemistry from ROS, other than O_2^-, which can lead to leukocyte adhesion. Oxidants from the Fenton reaction enhance production of the proinflammatory molecules platelet-activating factor and leukotriene B4, as well as increase the adhesion molecules P-selectin, E-selectin, and ICAM-1 (35). Oxidant activation of platelet activating factor and leukotriene B4 can activate nuclear factor-κB, which can result in synthesis of interleukin-8 and other adhesion molecules. Inhibition by NO of the oxidative chemistry in an analogous manner as described above for protection against peroxide-mediated toxicity also prevents the cascade of events that leads to increased leukocyte activation. Thus, the ability of NO to limit ROS chemistry prevents expression of different proinflammatory molecules and subsequently limits leukocyte activation and oxidative stress in ischemia–reperfusion-mediated tissue injury.

The role of NO in ischemia–reperfusion injury in the brain has been controversial (13). $ONOO^-$ toxicity has been invoked as the causative factor responsible for reperfusion-mediated tissue damage (2, 10). However, as discussed above, NO can be a powerful antioxidant. A recent study assessed the value of administering NO donor complexes at the time of reperfusion in both a focal and global brain ischemia–reperfusion injury in rat models (40). The major problem with systemic administration of most NO donors is that the resulting dramatic reduction of blood pressure places the patient at risk. This problem can be overcome by delivering NONOates with very short half-lives directly to the brain through intracarotid injection.

Infusion of DEA/NO in this manner, at the time of reperfusion subsequent to 5–20 min of ischemia, resulted in a dramatic decrease of the infarct area (40). A concurrent dramatic reduction in salicylate oxidation was also observed. Electrochemical monitoring of the brain NO levels showed that an initial NO burst (from eNOS) declined with time in the absence of NO donors. This reduction of NO production corresponded to an increase in salicylate oxidation. In the presence of the NO donor, NO levels were maintained throughout the experiment, and salicylate oxidation was reduced. From these data it was concluded that the primary function of NO in ischemia–reperfusion injury is as an antioxidant, which in part was responsible for reduction in infarct size. Therefore, NO donors have two benefits: restoration of blood flow and limiting oxidative stress in the injured tissue. Endogenous production of NO by eNOS limits infarct volume as do other antioxidants, such as Tempol (40, 47). These studies suggest that NO has a powerful antioxidant role in ischemia–reperfusion injury in the brain. Thus, for stroke, NO administration following treatment with antithrombotic agents may be beneficial.

Although the role of NO in ischemia–reperfusion injury represents acute tissue damage, atherosclerosis depicts a chronic disease that is mediated by oxidative stress. Although atherosclerosis has been proposed to be improved by the presence of NO, the positive role of NO in such diseases may be multifaceted, exerting protective effects chemically, biochemically, and physiologically (25, 61). Chemically, NO can prevent oxidative damage and limit lipid peroxidative chain propagation (25, 48, 49). This is similar to the factors that limit alkylhydroperoxide toxicity as discussed above. Ter-

mination of lipid peroxidation by NO may decrease low-density lipid oxidation whose products promote foaming macrophage and increase plaques. Furthermore, low-density lipid oxidation can lead to mast cell degranulation, which can induce further inflammation. Biologically, NO can inhibit platelet aggregation, prevent mast cell degranulation and leukocyte adhesion, and thereby is beneficial with respect to diseases that involve lipid oxidation (42). Thus, NO may have multiple effects in maintaining the health of the vascular wall, which may play a critical role in a number of acute and chronic injuries to tissue.

CONCLUSION

The ability of NO to abate oxidative stress plays an important role in both physiological and pathophysiological mechanisms involving properties at the chemical, cellular, and physiological levels. The relatively low concentration of NO required to be an antioxidant suggests that in addition to its involvement with cyclic GMP, this radical molecule serves to counterbalance oxidative stress. This balance between NO and oxidative stress provides an important regulatory mechanism in numerous physiological effects. Imbalance in this redox symbiotic relationship can lead to different pathophysiological conditions.

ABBREVIATIONS

AS, Angeli's salt ($Na_2N_2O_3$); CN^-, cyanide; CO_2, carbon dioxide; DEA/NO, $Et_2NN(O)NO^-$; DF, desferrioxamine; eNOS, endothelial nitric oxide synthase; GSH, glutathione; GSNO, *S*-nitrosothiolglutathione; H_2O_2, hydrogen peroxide; ICAM, intercellular adhesion molecule; $LOO^{\cdot}$, lipid peroxy radical; NO, nitric oxide; NO_2, nitrogen dioxide; O_2^-, superoxide; $ONOO^-$, peroxynitrite; RNOS, reactive nitrogen oxide species; ROS, reactive oxygen species; SIN-1, 3-morpholinosydnonimine; SNAP, *S*-nitroso-*N*-acetylpenicillamine; SNP, sodium nitroprusside; SOD, superoxide dismutase; XO, xanthine oxide/hypoxanthine.

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