Mechanisms of the pro- and anti-oxidant actions of nitric oxide in atherosclerosis

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Abstract

The association of nitric oxide (NO) with cardiovascular disease has long been recognized and the extensive research on this topic has revealed both pro- and anti-atherosclerotic effects. While these contradictory findings were initially perplexing recent studies offer molecular mechanisms for the integration of these data in the context of our current understanding of the biochemistry of NO. The essential findings are that the biochemical properties of NO allow its exploitation as both a cell signaling molecule, through its interaction with redox centers in heme proteins, and an extremely rapid reaction with other biologically relevant free radicals. The direct reaction of NO with free radicals can have either pro- or antioxidant effects. In the cell, antioxidant properties of NO can be greatly amplified by the activation of signal transduction pathways that lead to the increased synthesis of endogenous antioxidants or down regulate responses to pro-inflammatory stimuli. These findings will be discussed in the context of atherosclerosis. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

The concept that antioxidants play an important role in the atherosclerotic process is a natural corollary of the hypothesis that oxidative stress, or more recently nitrosative stress, contributes to vascular dysfunction and lesion development in the disease [1,2]. Recently, studies of the molecular mechanisms involved in combating oxidative or nitrosative stress have shown that the classification of molecules as antioxidants is not as simple as demonstrating a direct chemical or ‘scavenging’ reaction with a free radical or oxidant with additional roles as modulators of cell signaling pathways being suggested [3,4]. The antioxidant effects of any given molecule on atherogenesis can only be assessed when the biological properties of the products of the reactions between the antioxidants and radicals are considered. This is particularly evident in discussing the effects of nitric oxide (NO) in atherosclerosis. Both antioxidant and pro-oxidant roles for NO in the development of atherosclerotic plaques have been.
reported [2,5–15]. It is becoming clear that this paradox may be explained in part by the diverse biological effects of reaction products formed via the interactions of NO with different oxygen based radicals. In addition, an essential component of our understanding of the role of NO in vascular diseases is the identification of cell signaling pathways that are regulated by NO and impact significantly on the atherosclerotic process. These include signaling events that affect concentrations of cellular antioxidants, modulate cell proliferation or death and regulate the response of cells to pro-inflammatory stimuli. Some of these processes are mediated by activation of soluble guanylate cyclase (sGC) and these aspects have been discussed in a number of excellent reviews [16,17]. However, once the contributions from the sGC pathway have been accounted for it is clear that other NO-dependent cellular pathways contribute to the antioxidant properties of this molecule and serve as the focus of this review.

2. Reactions of NO with oxygen free radicals

The direct chemical interactions of NO with superoxide (O_2^-) or lipid peroxyl radicals (LOO^-) are of particular interest because they illustrate a molecular basis for some of the apparently antagonistic outcomes of similar chemical reactions when placed in a biological setting (Fig. 1). The reaction with O_2^- is rapid and facile (k=6.7×10^9 M/s), resulting in the formation of peroxynitrite (ONOO^-) [15,18]. It was originally thought that this reaction may be a mechanism to prevent superoxide dependent cytotoxicity. However, this view has not been supported by subsequent studies and the more complex picture that has emerged will be discussed in detail. Of particular note is the emerging concept that although ONOO^- is reactive and chemically unstable in molecular terms a biological half-life of seconds is not an impediment for a role as a cell-signaling molecule. A similar termination reaction occurs between NO and LOO^- (k=2×10^9 M/s) [19,20]. However, the biological effects of these two reactions are generally viewed as being opposite, with formation of ONOO^- usually associated with a pro-oxidant response and scavenging of LOO^- being an antioxidant response [6,8,9]. However, as discussed in this article, the biological outcomes of reactions of ONOO^- are not restricted to pro-atherogenic effects, and depending on the products of the reactions may be anti-atherosclerotic.

![Fig. 1. Inhibition of lipid peroxidation by NO. Initiation of lipid peroxidation occurs by abstraction of an allylic hydrogen atom from an unsaturated fatty acid. The rapid reaction between the corresponding carbon centered radical and oxygen forms a lipid peroxyl radical (LOO^-). Propagation occurs via the reaction with LOO^- and another fatty acid which forms a lipid hydroperoxide (LOOH) and re-generates a LOO^- . Lipid peroxyl radicals can also be formed by decomposition of LOOH in reactions catalyzed by transition metal ions (e.g. copper or iron) either free or in the form of heme proteins (e.g myoglobin and hemoglobin). The central role of LOO^- is thus apparent and scavenging of these radicals by antioxidants prevents lipid peroxidation. The classical LOO^- scavenging antioxidant is α-tocopherol which can react with one LOO^- to form the corresponding tocopheroxyl radical (α-toc). In turn, the α-toc can scavenge another LOO^- allowing a maximum of two LOO^- to be scavenged by α-tocopherol (in the absence of other co-antioxidants). The other product of these reactions however, is LOOH, accumulation of which renders the lipids to subsequent oxidation mediated by metal ions and heme proteins. NO also inhibits lipid peroxidation by reacting with LOO^- . The possible reactions that ensue are shown, however, a calculated stoichiometry of 2 moles of NO consumed per mole of LOO^- indicates that LONO or LONOO^- are reaction products. Importantly, inhibition of lipid peroxidation by NO is diffusion limited and does not result in LOOH formation which results in protection of the lipids to the potential oxidation mediated by metals and heme proteins. Based on the approximate concentrations of NO and α-tocopherol in vivo and the corresponding rate constants for reaction with LOO^- , calculations indicate that in vivo NO will be a more significant scavenger of LOO^- than α-tocopherol.](image-url)
2.1. Nitric oxide and oxygen based radicals

The relevance of ONOO\(^-\) formation to biological systems was recognized in the early 1990s and a role in the atherogenic process was suggested soon after [9–12,15]. Currently, ONOO\(^-\) is best considered as a prototype reactive nitrogen species (RNS) with a particular role to play in the early development of the atherosclerotic lesion [21]. These ideas have been derived from studies showing the presence of markers of reactions mediated by RNS in atherosclerotic lesions, namely 3-nitrotyrosine [10–12]. In addition to nitrination reactions ONOO\(^-\) is also an oxidant that can transform low-density lipoprotein (LDL) into a pro-atherogenic form [9,22–24]. This includes oxidation of both lipids and apoB resulting in a particle taken up by scavenger receptors that ultimately leads to cholesterol deposition in macrophages [25]. Furthermore, an early event in lesion development appears to be a shift in the production of NO and O\(_2\) in the vascular compartment in favor of O\(_2\) [26–28]. Important in the balance between NO and O\(_2\) are the mechanisms that lead to depleting or increased formation of either species. For example, O\(_2\) production is enhanced by the activation or transcriptional regulation of enzymes that produce this oxidant (e.g. NAD(P)H oxidases) and pathways capable of consuming NO appear to be up-regulated in vascular disease [28,29]. In either case increased ONOO\(^-\) mediated oxidative damage is likely to occur and accumulate. It should be noted however, that recent evidence indicate that a number of mediators can mediate nitrination and oxidation reactions in atherosclerotic lesions including reactions of enzymes such as myeloperoxidase with nitrite [30,31].

The reaction of NO with LOO\(^-\) is similar to the reaction with O\(_2\) in that it is also a termination reaction. In this context, this reaction is viewed as beneficial as it results in cessation and inhibition of lipid peroxidation reactions [6–8,20]. This is analogous to the classical antioxidant effects of \(\alpha\)-tocopherol towards inhibition of LDL oxidation, although as discussed later, on a molar basis NO is more efficient.

The termination reactions of NO with free radicals can also occur with radical species that are intermediates in enzymatic processes. In this case NO has the potential to regulate the formation of pro-inflammatory products from enzymes such as the lipooxygenases and cyclooxygenases [32]. The reaction mechanisms between NO and peroxyl radicals and the effects these have on lipid peroxidation are discussed in the following section.

3. Scavenging of lipid radicals by NO and seeding peroxides in lipoproteins

Oxidation of lipids is key in the atherosclerotic process since primary oxidation products, the lipid peroxides, and secondary oxidation products, such as the aldehydes are found in human atherosclerotic lesions [33–35]. This also suggests that both termination and initiation reactions are occurring in the lesion. A characteristic of free radicals is that termination reactions in which two free radicals react together to form a non radical species are often extremely rapid, a fact highlighted by the diffusion limited rate constants for the reaction between NO and either O\(_2\) and peroxyl radicals [18,19]. Termination reactions are the most likely control point for suppression of lipid oxidation by NO in the extracellular environment. Lipid oxidation reactions must start by the formation of a radical species from a non-radical fatty acid precursor. This can be achieved by abstraction of a H atom from the bis-allylic bond of an unsaturated fatty acid to form an alkyl radical (L\(^-\)) (Fig. 2) [36]. In principal NO could react with the alkyl radical. However, oxygen also reacts with this species at diffusion limited rates and given the substantially higher (10–100 times) concentrations of oxygen relative to NO, it is not likely that NO can efficiently compete for reaction with the alkyl radical. A peroxyl radical (LOO\(^-\)) is thus formed and it is central to propagation reactions that lead to extensive oxidative damage to lipids. An initiation reaction can also occur with preformed lipid peroxides that could originate from the reaction of enzymes such as 15-lipoxygenase [37]. In this case the decomposition of pre-existing lipid hydroperoxides by transition metals or heme proteins occurs forming both alkoxyl and peroxyl...
radicals (Fig. 2) [38–40]. This reaction is the basis of the frequently used copper oxidation of low density lipoprotein and this reaction is inhibited by NO [8,41].

After formation of LOO⁺ this species is capable of regenerating itself in an autocatalytic reaction known as lipid peroxidation. This is the amplification phase of the reaction and in biological systems this is usually terminated by antioxidants that donate a hydrogen atom or an electron such as α-tocopherol [42]. Although this mechanism suppresses lipid propagation reactions, a lipid peroxide and antioxidant free radical are side products (Fig. 1). This can present a problem because the slow accumulation of these so called seeding lipid peroxides renders a lipoprotein progressively more susceptible to metal and heme protein-dependent oxidation and the antioxidant radical can reinitiate peroxidation if not scavenged.

The importance of these low concentrations of ‘seeding’ lipid peroxides is that they may underlie the finding that LDL isolated from risk groups for atherosclerosis are more susceptible to oxidation by copper (see references in Ref. [43]). Nitric oxide is quite different to α-tocopherol in this respect and reacts with LOO⁺ in two steps. Initially NO undergoes a termination reaction with organic peroxyl radicals to form a non radical species that has some of the characteristics of peroxynitrites [19]. This unstable intermediate then consumes NO in an additional step before formation of what is presumed to be a stable organic nitrate. This mechanism explains the finding that two NO molecules are required to scavenge one LOO⁺ [20].

4. Scavenging of lipid peroxyl radicals: NO verses α-tocopherol

As mentioned above two molecules of NO are consumed for every LOO⁺ radical scavenged [20]. This is less favorable than α-tocopherol which can scavenge two LOO⁺ per molecule. However, this disadvantage is offset by the fact that the reaction of NO with a LOO⁺ is approximately 10 000 times faster than the same reaction of α-tocopherol with lipid peroxyl radical [20,42]. The net result is that a low concentration of NO is far more efficient in scavenging LOO⁺ with very little ‘leak’ and chain propagation occurring [7]. Computer simulations suggest that at steady state concentrations of approximately 30 nM NO would lead to an equivalent extent of inhibition of lipid peroxidation as 20 μM α-tocopherol [20]. This has important ramifications in the context of a vessel where NO is continually generated and is consistent with the observation that in the presence of an NO donor, loss of α-tocopherol is ‘spared’ in an experimental model of LDL oxidation [44]. A further advantage of NO as an antioxidant over α-tocopherol is that its availability is not limited by the transport of a hydrophobic moiety via the lipoprotein; NO is freely diffusible and partitions into the lipid phase [45].

5. The reaction of NO with peroxyl radicals in lipoxygenase

A number of enzymes use controlled oxygenation reactions to produce second messengers and recent data suggests that at least one of these, 15-lipoxygenase, plays an important role in the atherosclerotic process [46,47]. Several pieces of evidence indicate a role for this enzyme in promoting atherosclerosis. For example, in vitro incubation of lipoxygenase with LDL results in oxidative modification of the latter and this has been suggested to be a mechanisms for cell-dependent oxidative damage to the lipoprotein [48,49]. Also, lipoxygenase mRNA was found to co-localize with epitopes for oxidized protein–lipid adducts in atherosclerotic lesions [50]. Furthermore, apoE-deficient mice in which the lipoxygenase gene was disrupted failed to show significant development of atherosclerotic lesions compared to control animals [47]. Analysis of the time course of lesion formation indicated that lipoxygenase has a key role in initiation of the disease. The catalytic cycle for lipoxygenases is now understood in some detail and the insertion of peroxide group into a fatty acid substrate involves an intermediate alkyl and peroxyl radical bound to the iron atom in the active site [51]. This process then has the potential for interaction with NO at either the metal center or the fatty acid derived radical intermediates. Evidence for both reactions has been presented in the literature but the interactions with the metal center can only be detected at high non-physiological concentrations of NO [52]. Two other sites of interaction of NO were recently identified involving lipid and possibly protein intermediates [32]. The activation of 15-lipoxygenase requires a lipid peroxide to bind to the protein and this is associated with oxidation of the inactive ferrous non-heme iron center to the active ferric form [37]. The lipid peroxide is not changed during the process and this suggests a protein radical is formed during the activation step. In support of this finding a molecule of NO is also consumed but this does not appear to result in inhibition. In the catalytic cycle itself, NO is proposed to react with the LOO⁺ intermediate returning the enzyme to the inactive ferrous form. The potential significance of this reaction for the early stages of atherosclerosis is that the consumption of NO is competitive with activation of sGC [32] and it is hypothesized that this may contribute to the observed loss of NO-dependent regulation of vessel tone during atherogenesis [26,27]. Although the 15-lipoxygenase hypothesis has been developed in the context of LDL oxidation these data serve to emphasize the importance of cell signaling pathways regulated by free radicals in the atherosclerotic process. It seems likely that an important
contributions to the development of the atherosclerotic process by 15-lipoxygenase is the loss of NO-dependent cell signaling in the vasculature.

6. The reaction of NO with $O_2^-$ to form ONOO$^-$

Oxidized lipid products have been detected by a wide variety of techniques in atherosclerotic lesions and are apparently co-existent with antioxidants such as $\alpha$-tocopherol and ascorbate, which inhibit lipid peroxidation in vitro promoted by most oxidants [53]. Neither of these antioxidants have a significant effect on lipid peroxidation initiated by the enzymatic action of 15-lipoxygenase. However, the product from lipoxygenase oxidation of fatty acids is a specific stereoisomer that is found to be only modestly elevated in human atherosclerotic lesions over the random products formed from non-enzymatic lipid peroxidation [34]. Clearly another oxidant (or oxidants) is also contributing to peroxidation of lipids in the pro-inflammatory environment of the developing lesion. Peroxynitrite is particularly interesting in this respect, since it is one of the few oxidants capable of initiating lipid peroxidation in the presence of biological antioxidants such as $\alpha$-tocopherol and ascorbate [22–24]. A series of studies demonstrated that ONOO$^-$ can oxidize LDL to a pro-atherogenic form with the concomitant oxidation of proteins and lipid [9,22–24]. Furthermore, ONOO$^-$ can deplete levels of $\alpha$-tocopherol and by forming seeding lipid hydroperoxides prime LDL for subsequent oxidative damage mediated by transition metal ions and heme proteins [24].

The relative rates of synthesis of NO and $O_2^-$ appear to vary dramatically during the course of the atherosclerotic process as does the site and source of production of these two free radicals. This is important since it has been shown that a critical parameter in determining the extent of oxidation and nitrination reactions in biological systems is the balance between NO and $O_2^-$ [6], which is dependent upon both processes that produce and remove these species. A slight excess of NO appears to be capable of suppressing lipid oxidation reactions mediated by the simultaneous formation of NO and $O_2^-$. As a therapeutic strategy the addition of exogenous NO will restore the balance in favor of NO and inhibit the amplification due to propagation of lipid peroxidation reactions. In support of this concept NO donors protect endothelial cells from oxidative damage elicited by oxidized LDL and as discussed below this is a potential detoxification mechanism for RNS [7,54].

Since the relevance of ONOO$^-$ formation to biological systems was realized, many studies have documented the cytotoxic potential of this RNS [55]. However, an alternative point of view on the role of NO/$O_2^-$ interactions in vivo has been expressed by some investigators. The biological lifetime of ONOO$^-$ is relatively short (less than a second) and the most quantitative route of ONOO$^-$ decay is to nitrate, a biologically inert molecule [56]. These facts have led to the suggestion that in vivo NO has antioxidant effects by efficiently scavenging $O_2^-$ and inhibiting hydrogen peroxide ($H_2O_2$) production. Although nitrate is a significant reaction product, nitisation, nitrination and oxidation of biological molecules by ONOO$^-$ has been demonstrated in many biologically relevant systems (see below). The latter reactions occur with a relatively low yield, but the high sensitivity of the biological responses to these modifications provides mechanisms by which ONOO$^-$ can affect cell function independent of modulation of NO, $O_2^-$ or $H_2O_2$ concentrations. In fact, recent studies suggest a cytoprotective role for NO and $O_2^-$ interactions via the production of ONOO$^-$ [57,58]. These are discussed in more detail later.

7. Formation of NO donors from nitration and nitrosation reactions of ONOO$^-$

The direct addition of preformed ONOO$^-$ to biological systems has shown an interesting spectrum of effects that cannot be explained by simple oxidation reactions. Early studies demonstrated that in vascular preparations addition of ONOO$^-$ and the ensuing nitrination and nitrosation reactions resulted in the formation of a spectrum of compounds with NO donor properties [59–63]. Thus ONOO$^-$ elicits vessel relaxation and inhibits platelet aggregation through an NO-dependent mechanism [59–62,64]. The particular NO donor formed depends upon what is available for ONOO$^-$ to react with. The molecular identification of these species has shown that with thiols an S-nitro or S-nitroso group can be added to the molecule [59–63]. For example with glutathione, S-nitrosoglutathione is one of the products formed. With alcohol functional groups the product is an organic nitrate or nitrite [60,65]. With glycerol the mononitritated metabolites of the clinically used nitrovasodilator, glycerol trinitrate were identified in addition to a novel glycerol mononitrite as products of the reaction with ONOO$^-$ [65]. Such compounds can explain many of the systemic effects of introduction of ONOO$^-$ into the circulation of experimental animals including the rapid development of tachyphylaxis or tolerance [66,67].

Formation of NO donors is of particular interest since they potentially offer a mechanism for the production of NO at a cell surface within an extracellular environment that is pro-inflammatory and pro-oxidant. S-nitrosothiols appear to be central in these effects since they can be formed directly by ONOO$^-$, but also have been proposed as intermediates in the NO donor actions of organic nitrates and nitrates (Fig. 2). Furthermore, emerging data indicate that cell specific mechanisms that reduce S-nitrosothiols to
NO exist [68–70]. In addition, S-nitrosation (and subsequent S-thiolation) of critical cysteine residues can modulate protein function and examples of such mechanisms in regulating specific cell signaling pathways have been reported [71–73] (Fig. 2). Thiol are important mediators of ONOO− reactivity because of their relatively high concentrations in both the extra- and intracellular compartments and also because they are important modulators of S-nitrosothiol biology [69,74]. It follows that the thiol status in the vasculature may then be a factor in determining the response to ONOO−. In this respect a recent study has shown that in hypercholesterolemic rabbits the main intracellular thiol antioxidant, glutathione (GSH), is depleted and the detrimental effects of ONOO− on vascular function accentuated [75].

It should be noted that not all NO donors formed from the reaction with ONOO− require metabolism to release NO. A compound is formed from the reaction with urate appears to decompose in solution at a slow rate with the release of NO [76]. It is important to note that while the chemical efficiency of the nitration and nitrosation reactions is probably only a few percent, in biological terms the effects are amplified if NO is a metabolic end product. Indeed low nM concentrations of NO are generally sufficient to activate the soluble guanylate cyclase pathways and this concentration of NO can be readily achieved from the reactions of ONOO− with thiols and other molecules capable of acting as precursors to NO donors [59,65,77].

The biological dominance of the nitration and nitrosation reactions over oxidation is a current theme emerging in our understanding of the effects of ONOO− in physiological systems. It is also concordant with the biochemical observations that indicate reaction of ONOO− with carbon dioxide forms an intermediate that favors nitration reactions over oxidation [78,79]. The high concentrations of carbon dioxide in vivo suggest that it will significantly dictate the biological effects of ONOO−. The potential biological advantage, and hence a protective effect of ONOO−, may derive from the fact that the ONOO− derived NO donors can be transported into vascular smooth muscle cells or platelets and be metabolized to release NO leading to activation of soluble guanylate cyclase. In support of this argument a recent study has shown that a potential cardioprotective effect of ONOO− at low concentrations of 0.2–2 μM Peroxynitrite exposure decreased P-selectin expression and preserved endothelial cell function [58]. Furthermore, formation of ONOO− in endothelial cells subjected to shear stress, an antiatherogenic stimulus, was implicated in the activation of the mitogen activated protein kinase, JNK [57]. Whether the mechanisms involved in ONOO− dependent cell signaling include S-nitrosation or nitration of specific proteins remains unclear, but are implicated by findings demonstrating that NO dependent modification of critical protein thiols affects function. Examples relevant to cell signaling include p21 ras and AP-1 [71]. Similar effects were observed in platelets where the NO-dependent antiplatelet properties were evident at low concentrations of ONOO− with oxidation of thiols only detectable at much higher levels [61]. It is important to recognize that in cell signaling intracellular location and the formation of discrete signaling domains composed of complexes of many proteins are known to be important. For example, in the case of eNOS the association of the enzyme with subcellular structures known as caveoli modulate NO production in response to a wide range of agonists [80]. The significance of the specific cellular location of the NOS enzymes to redox signaling is an area that we know relatively little about. However, if reactive molecules such as ONOO− play a role in signal transduction having the ‘receptor’ closely located to the site of formation may be a mechanisms to endow specificity to the signaling cascade.

8. NO-dependent regulation of cell signaling pathways that could inhibit atherosclerosis

As a further elaboration of the hypothesis that thiol content is a major determinants of NO function in the vasculature, it has been shown NO can induce GSH synthesis in both endothelial and vascular smooth muscle cells [81,82]. This is a potentially important supplement to the antioxidant functions of NO associated with scavenging of oxygen radicals, and can have the effect of short term exposure to NO having long term effects on vascular function. Nitric oxide increases intracellular GSH concentration by induction of γ-glutamylcysteine synthetase (GCS), the rate-limiting enzyme of glutathione synthesis, as well as cellular cystine uptake [81–83]. Thus both determinants of GSH synthesis, the activity GCS and the supply of limiting substrate cysteine, are increased concomitantly. In vascular smooth muscle cells GSH synthesis is increased through transcriptional induction of both the heavy and light GCS subunits [81]. This process was shown to be independent of the classical cGMP-soluble guanylate cyclase signaling pathway. This is important since in vascular lesions, smooth muscle cells adopt a synthetic phenotype and are incapable of mediating cGMP-dependent signaling due to the down-regulation of the binding protein for cGMP, protein kinase G 1α [84]. That GSH synthesis is dependent on NO is further evidenced in vivo in a study, in which renal GCS activity is down-regulated by 50% by chronic NOS inhibition in rats [85].

Glutathione appears to regulate NO production in a reciprocal manner. In purified enzyme preparations of iNOS and nNOS, GSH is needed for full enzyme activity [86,87]. N-acetylcysteine (NAC), a thiol antioxidant and a GSH precursor, has been shown to enhance interleukin-1β-induced nitric oxide synthase expression in the vascular smooth muscle cells [88]. Furthermore, endothelial NO synthesis in impaired in glutathione-depleted endothelial cells [89].
Many characteristic pathophysiological findings in atherosclerosis appear to be dependent on cellular thiol status. Impaired endothelium-dependent vasodilation in patients with coronary artery disease can be reversed by a non-thiol cysteine prodruk, l-2-oxo-thiazolidine carboxylate (OTC), which is known to increase vascular GSH concentration [90,91]. Similar effects of ascorbic acid may be accounted for the sparing of intracellular glutathione [92,93]. Vascular smooth muscle cell proliferation induced, e.g. by growth factors such as PDGF, appears to be mediated by ROS and is inhibited by NAC [94]. NO, in turn, inhibits vascular smooth muscle cell proliferation [95]. Nitric oxide inhibits activation of the redox sensitive transcription factor NF-κB in response to proinflammatory cytokines which in turn prevents transcription of chemokines such as monocyte chemoattractant protein-1 (MCP-1) and the adhesion molecules VCAM-1 and ICAM-1 [96–98]. This is important in the early stages of atherogenesis where monocyte recruitment and binding to the endothelium is mediated by these inflammatory mediators. As thiol antioxidants appear to have similar inhibitory effects on NF-κB activation and subsequent induction of inflammatory genes, it is possible that NO functions by altering intracellular thiol status [99,100]. Indeed, marked increases in monocyte infiltration into coronary vessels as well as in MCP-1 expression and NF-κB activation caused by chronic inhibition of NO synthesis in rats are effectively reversed by intraperitoneal administration of NAC [101].

Taken together, there appears to be a close interaction between NO and GSH synthesis in the vascular wall. Furthermore, NO seems to inhibit many of the characteristic findings of atherosclerosis, such as monocyte recruitment and vascular smooth muscle cell proliferation in a redox-dependent manner. These findings strengthen the idea that NO-dependent modulation of redox sensitive signal transduction pathways is important in the anti-atherosclerotic effects of this free radical.

9. Summary

It now seems likely that NO complements lipophilic antioxidants present in lipoproteins in controlling oxidative and nitrosative stress in the vascular wall through both direct chemical mechanisms and transcriptional control of cellular antioxidants. From the perspective of redox cell signaling, which requires some increase in oxidative stress to initiate cytoprotective pathways in response to inflammation, it make sense to use an antioxidant that can be controlled at the cellular level. The finding that reactive nitrogen species such as peroxynitrite may play a role as cell signaling molecules and that this may be mediated by several interrelated mechanisms is both provocative and challenging. Before this can be accepted a number of missing elements need to be defined. For example, if nitration of tyrosine or S-nitrosoation of thiols is analogous to phosphorylation or S-thiolation than specific receptors and enzymes to reverse these processes need to be sought. Notwithstanding the incomplete nature of our knowledge in this area, the current picture that is emerging suggests that the role of NO in the atherosclerotic endothelium may be understood by focussing on mechanisms related to the effects of reactive nitrogen species on cell signaling pathways.

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