Nitric oxide- Not enough, Too Much & How it Goes Bad

Nitric oxide (•NO) is a very vogue molecule first discovered in 1977. In 1992 it was declared the molecule of the year and in 1996 the Nobel Prize was awarded to the discoverers of its effects upon the vascular system. •NO has been implicated in a wide range of important physiological events such as heart attack, headache, athletic performance and erections.

Athletes have been using nitrate and arginine supplements as precursors to nitric oxide so they can ensure higher levels of performance. There is little talk of caution about too much •NO among this community, indeed, the consensus seems to be “more is better”. Certainly there are problems when •NO is deficient such as poor blood flow in extremities (cold hands) and reduced blood flow to the heart which can lead to heart attacks. There are, however, dangers associated with high levels of •NO. Too much •NO can lead to headaches, and after the body has habituated to high levels of nitrates, withdrawal can lead to heart attack. Clearly, whatever is done to improve the levels of nitric oxide, it has to be done consistently to avoid rapid withdrawal syndromes. The nitroglycerin industry flourished from 1900, exposing workers to high levels of organic nitrites; the phenomena of nitrate tolerance was recognized by the onset of 'Monday disease' and of nitrate-withdrawal/overcompensation by 'Sunday Heart Attacks'.[1] (O'Donnell & Freeman, 2000)

•NO is a reducing radical important for dilation of blood vessels. A radical is a molecule with an unpaired electron, hence the inclusion of a superscript dot (•) before or after the chemical formula. This unpaired electron makes the molecule very reactive. Most of the radicals we discuss commonly are oxidizing radicals, and this is what most people mean when they describe “free radicals” and their health hazards, since uncontrolled non-enzymatic radical production is generally an unhealthful thing. •NO is different, instead of taking electrons from other molecules (oxidation) it gives up an electron in reactions (reduction). The oxidation of nitric oxide terminates its action after it is released. Every neurotransmitter and hormone needs to have a mechanism for the termination of its function, for example, acetylcholine is broken down by acetylcholine esterase, dopamine is removed from the synapse by reuptake transporters, etc.

•NO is eliminated by oxidation but this becomes too rapid in inflamed people. The oxidation associated with inflammation is a burgeoning health issue that results from the poor quality of the modern diet. This means that otherwise adequate levels of •NO signal now fail to achieve the proper impact in inflamed individuals. The simple answer would seem to be inducing an increase in the levels of •NO.

[1] http://circres.ahajournals.org/content/88/1/12.long

Why More isn't Better

An analogy to trench warfare describes the problem of nitric oxide. Taking large quantities of precursors and stimulating •NO production would seem to be good. If •NO has a reduced half-life due to inflammation, just throw more troops across no man's land and the number reaching the target will return to normal.

This tactic will indeed result in greater transmission of •NO signaling, though this can create a dynamically unstable situation seesawing between overdose and withdrawal. There is another aspect of too much nitric oxide- the threat of oxidation products from •NO that spawn a myriad of uncontrollable radicals. In the analogy of trench warfare- even if you can afford to put a great number of troops across no man's land, the injured and dead that result have their own unwanted consequences of rot and disease.

This problem is described by O'Donnell and Freeman:
"Alternatively, if generated at elevated levels, for example, after inducible nitric oxide synthase expression in inflammation, •NO can be converted to prooxidant species, such as peroxynitrite ($ONOO^-$) and nitrogen dioxide ($^•NO_2$), that can potentiate inflammatory injury to vascular cells." [2](O'Donnell & Freeman, Interactions Between Nitric Oxide and Lipid Oxidation Pathways, 2010)

The implication of this is that having more •NO produced to overcome the destruction of •NO by an inflamed system can actually create new classes of radicals that damage the endothelial lining of the vascular system. Since these endothelial cells generate •NO the result is negative feedback where making more •NO actually destroys the tissues that produce it. This tactic provides diminishing returns from supplementation along with greater risk of vascular diseases such as atherosclerosis.

Leopold and Loscalzo describe the process of vascular damage by oxidizing radicals:

"In the vasculature, reactive oxidant species including reactive oxygen, nitrogen, or halogenating species, and thiyl, tyrosyl, or protein radicals, may oxidatively modify lipids and proteins with deleterious consequences for vascular function. These biologically active free radical and non-radical species may be produced by increased activation of oxidant-generating sources and/or decreased cellular antioxidant capacity. Once formed, these species may engage in reactions to yield more potent oxidants."[3] (Leopold & Loscalzo, 2010)

Reactions of the Oxidizing Agents Generated by •NO

Fatty Acid Nitration

[2] http://circres.ahajournals.org/content/88/1/12.long
[3] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2797369/

Freeman et. al. describe the many downstream effects of •NO oxidation quite well:

"The biochemistry of fatty acid nitration stems from the reactions of •NO, •NO-derived oxides of nitrogen (e.g. nitrogen dioxide ($^{\bullet}NO_2$) and peroxynitrite ($ONOO^-$)), and oxygen-derived inflammatory mediators (e.g. superoxide $^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), and lipid peroxyl radicals ($LOO^{\bullet}$)). Multiple mechanisms induce biomolecule nitration, with this redundancy supporting the concept that nitration reactions transduce •NO signaling and tissue inflammatory responses. The formation of secondary •NO-derived species and the subsequent reactions that mediate biomolecule nitration are dictated by •NO concentration; oxygen tension; site of generation; local concentrations of targets, catalysts, and scavengers; and partitioning between hydrophobic and hydrophilic compartments. These factors also reflect cell metabolic and inflammatory status and ultimately govern relative extents of target molecule oxidation, nitrosation, and nitration.
…
In addition to reaction with thiols, $ONOO^-$ reaction with carbon dioxide (CO_2) predominates in tissues, yielding $ONOOCOO^-$. In an inflammatory milieu, both protonated $ONOO^-$ (ONOOH) and $ONOOCOO^-$ yield $^{\bullet}NO_2$ along with •OH and $^{\bullet}COOO^-$ as products that can support biomolecule nitration reactions.
…
Aerobic Reactions of •NO—Because of a small molecular radius and uncharged nature, •NO is lipophilic and can concentrate up to 20-fold and more readily react with the molecular O_2 that also preferentially partitions into a hydrophobic milieu. This molecular "lens" effect induced by •NO and O_2 concentration in hydrophobic compartments can accelerate •NO oxidation by 2–3 orders of magnitude. This lens effect thus promotes the formation of secondary •NO-derived species from O_2 ($k = 2 \times 106\ m^{-2}\ s^{-1}$), including $^{\bullet}NO_2$"

Figure 1 from this publication (below) gives a good overview of the nitric oxide derived radicals and their interactions with fatty acids.

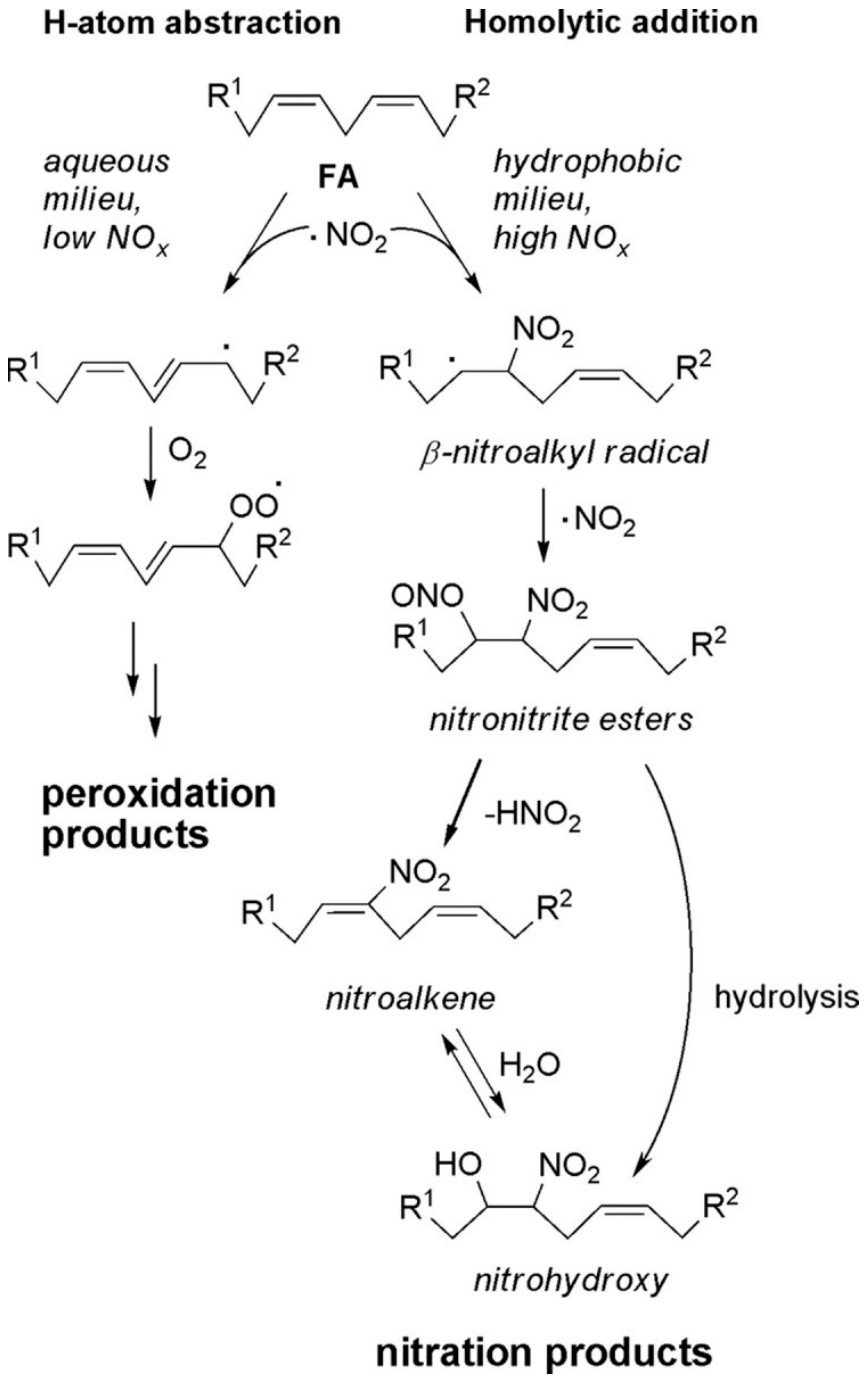

Figure 1- Mechanisms of fatty acid nitration and oxidation by nitric oxide-derived species[4] (Freeman, Baker, Schopfer, Woodcock, Napolitano, & D'Ischia, 2008)

Tyrosine nitration- Cellular Signal, or an Indicator of Oxidative Inflammatory Responses?

[4] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2414282/

In addition to the interactions between nitrogen radicals and fatty acids, there are further interactions between the amino acids in proteins and nitrogen radicals. The nitration of proteins can destroy their structural integrity and/or their enzymatic functions. Recently, the signaling potential of tyrosine nitration has been discovered and it modulates many cellular activities. Given that this is a signal, the question becomes whether this signal is intrinsic to proper cellular functioning, or is it an "emergency signal" that has no beneficial role in the absence of damage and the need to respond to that damage?

Many oxidizing radicals have been discovered which transmit signals and activate specific receptors. The question is whether these radicals are an essential part of the signaling repertoire of the cell, or are these cellular responses more like a fire engine. i.e. is this only really useful in the event of dysfunction, but unnecessary if there is some better way of preventing or putting out the fire?

Here is a description from Schopfer et. al that discusses the role of •NO-mediated oxidative inflammatory reactions:

"Tyrosine nitration is becoming increasingly recognized as a prevalent, functionally significant post-translational protein modification that serves as an indicator of nitric oxide (•NO)-mediated oxidative inflammatory reactions. Nitration of proteins modulates catalytic activity, cell signaling and cytoskeletal organization. Several reactions mediate protein nitration, and all predominantly depend on •NO and nitrite-dependent formation of nitrogen dioxide, a species capable of nitrating aromatic amino acids, nucleotides and unsaturated fatty acids." [5] (Schopfer, Baker, & Freeman, 2003)

How the Naked Electron Defends •NO as it Travels Through the Bloodstream

The highly mobile and rapidly reacting naked electrons in Heliopatch are directly attracted by the most potent radicals and oxidizing agents. Some oxidizing agents such as nitroperoxyl can travel through the body and suddenly become reactive radicals, but as soon as they do, they will draw the naked electron and be neutralized. This process of electrons flowing toward oxidizing radicals isn't like aiming a gun, it is a much more passive process similar to the way that water flows downhill. This makes it immediately effective and incredibly potent no matter what form an oxidizing agent takes and what it does once it becomes a radical. The naked electron is a perfectly matched opposing force to oxidizing radicals; the faster and more powerful they are, the more rapidly they are neutralized and at a greater distance. In the analogy of trench warfare, this is a means of preventing the soldiers going across no man's land from being destroyed en route, so there is no need for over production and no casualties on the battlefield to cause additional dysfunction.

[5] http://www.ncbi.nlm.nih.gov/pubmed/14659696

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