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REVIEW

Nitric oxide, health and disease

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Summary
Nitric oxide is a gaseous substance which possesses many important physiological characteristics ranging from its action as a natural immune mechanism to endothelial control of blood pressure. However, it can also generate nitrogen reactive species (peroxynitrite and others), which are involved as a cause of or a consequence of many diseases. This article updates and summarizes the physiological and pathophysiological roles of nitric oxide.

Key words: peroxynitrite*;* cancer; cardiovascular diseases; phagocytosis; diabetes mellitus; infection

INTRODUCTION

The internal milieu of the cell depends on an acid pH electrolytic balance as well as many physiological factors. One important aspect of cell and body protection is the nitrosative balance, i.e. the adequate dynamic homeostasis between the synthesis and release of nitric oxide ($NO^{\bullet}$) and reactive nitrogen species [RNS, especially peroxynitrite ($ONOO^{\bullet}$)] by cytosolic organelles, and their scavenging by the antioxidant defense mechanisms. When this equilibrium is broken by the overproduction of RNS, and/or decreased antioxidant levels (by failure in synthesis or decreased nutritional intake and bioavailability), body tissues and cells are suddenly affected by the pathological consequences of nitrosative stress (Ridnour et al. 2004, Tavazzi et al. 2007).

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BIOCHEMICAL ASPECTS OF NITRIC OXIDE AND RNS

Nitric oxide ($NO^{\bullet}$) is an important modulator of blood vascular tone. It is also known as the endothelial-derived-relaxing factor (EDRF), and can form free radicals capable of peroxidizing the LDL, proteins, and many other biomolecules (Hog et al. 1993, Ferrari 2001), according to the following reactions:

$2NO^{\bullet} + O_2 \Rightarrow 2NO_2^{\bullet}$ (nitrogen dioxide radical)
$NO_2^{\bullet} + L\text{-}H \Rightarrow L^{\bullet} + HNO_2$ (nitrous acid)
$NO^{\bullet} + O_2^{\bullet -}$ (superoxide) $\Rightarrow ONOO^{\bullet}$ (peroxynitrite, a stronger reactant).

Superoxide anion sources for peroxynitrite synthesis comprise the mitochondrial respiratory chain, xantine-oxidase, uncoupled eNOS, and NADP(H)oxidases (Pacher and Szabó 2008). Peroxynitrite induces activation of poly (ADP-ribose) polymerase (PARP-1) which in turns activates the expression of inflammatory genes and increases the risk of cell death by depleting NAD^+ and ATP cytosolic stores (Yu et al. 2006, Pacher and Szabó 2008).

Arginine is the substrate for NO synthesis by four isoforms of Nitric Oxide Synthases (NOS): endothelial nitric oxide synthase (eNOS, a Zn-metalloenzyme), neuron NOS (nNOS), inducible NOS (iNOS) (Lamas et al. 1998), and the mitochondrial isoform (mtNOS) (Giulivi et al. 1998, Lamas et al. 1998). NO can suppress cell death in many cell systems and models (Ferrari 2000). Beyond those effects, NO inhibits mitochondrial enzymatic systems, such as cytochrome c oxidase and mitochondrial complex I. The inhibition of mycobacterial lung infection and cancer proliferation (through suppression of polyamine synthesis) and the production of cytotoxic peroxynitrite, are also important roles played by NO (Lamas et al. 1998, Kondo et al. 2002).

NITRIC OXIDE AND IMMUNE DEFENSE

NO and peroxynitrite are important immunomodulatory agents against infectious and parasitic pathogens (Missal et al. 2006, Forlenza et al. 2008). Macrophage cytosolic L-arginine is converted by nitric oxide synthase-2 (NOS2) into nitric oxide which potentially enhances the cytotoxic and antimicrobial activity of mucosal phagocytes (MacMicking et al. 1997, Wang and Kuo 2001, Iovine et al. 2008). Interestingly, nitric oxide increases protection by inducing the gel-forming glycoproteins mucins which improve the defence against mucosal pathogens (Linden et al. 2008). It has been suggested that the immunological roles of NO are mediated by Interferon-gama (IFN-γ) release, and target inflammatory and nitroxidative gene expression (Prasanna et al. 2007).

PHYSIOLOGICAL ROLES OF NITRIC OXIDE

Nitric oxide acts as a potent vasodilator which can improve myocardial function during ischemic injury and also has positive effects on acute ischemic and hemorrhagic stroke patients (Brunner et al. 2003, Rashid et al. 2003). The regular practice of aerobic exercise stimulates NOS enzymes releasing substantial amounts of NO which decisively contribute to the improvement of arterial blood pressure control through the relaxation of the vascular smooth muscle cells (Kingwell 2000, Roberts et al. 2002, Wilcox 2005). Exercise training enhances NO, GSH, GSH/GSSG ratio and decisively contributes to decreased blood oxidants and lactate (Husain et al. 2003). Even in well controlled diabetic patients there is a NO deficiency which could impair endothelial vasodilation (Woodman et al. 2006). Interestingly, the same study reported that some diabetic patients had increased HDL levels which worked as a compensatory mechanism in blood pressure control. It should be noted that regular practice of physical exercise can also increase HDL cholesterol levels, thus decreasing atherosclerosis risk (Nordstrom et al. 2003). Following the same approach it should be noted that exercise and dietary restriction enhance NO bioavailability improving blood glucose and lipids, inflammation, and blood pressure control – components of the metabolic syndrome (Ferrari 2007, 2008). The physiological protective actions of nitric oxide are listed in Table 1.

PATHOPHYSIOLOGICAL ASPECTS OF NITRIC OXIDE AND REACTIVE NITROGEN SPECIES

NO has seemingly incompatible roles in health and disease. It is used to manage pulmonary hypertension in neonates and in patients with acute distress syndrome, but in certain doses NO may stimulate inflammation and peroxidation in the lung, also inducing oxidative DNA damage (Weinberger et al. 2001). In part, this could be explained by the different NOS isoforms. In the brain and the endothelium, iNOS has deleterious effects and eNOS has protective vasodilatory effects, but iNOS activation in the liver has also protective effects. Research has pointed out that individuals with G894T and T-786C polymorphisms on the eNOS gene have increased risk of atherothrombosis (Loscalzo 2003, Spoto 2007).

When NO reacts with iron, a nitrosyl compound is formed which can trigger deleterious changes in many sulphur-containing molecules, such as secondary amines, phenolics, and thiols, as represented below (Nappi and Vass 2002):

$NO + Fe^{3+} \rightarrow NO\text{-}Fe^{3+}$

$NO\text{-}Fe^{3+} + R\text{-}SH \rightarrow RSNO + Fe^{2+} + H^+$

Table 1. **Physiological roles of nitric oxide**

NO protects against superoxide and H_2O_2 released by xanthine-oxidase toxicity (Wink et al. 1995)	Adriamycin-induced cardiotoxicity is mediated by a superoxide radical which is abolished by both NO and SOD (Cole et al. 2006)
Vasodilation and blood pressure control, avoiding atherogenesis (Husain et al. 2003, Nordstrom et al. 2003)	Exercise-induced vasodilation (Kingwell 2000, Roberts et al. 2002)
Anti-bacterial effects: NO decreased *Neisseria meningitidis* (Dyet and Moir 2006) and *Coxiella burnetii* infections (Brennan et al. 2004); NO inhibited both growth and listeriolysin O production contributing to intracellular macrophage destruction of *L. monocytogenes* (Ogawa et al. 2001)	Anti-leishmaniasis: NO can kill promastigotes and amastigotes of *Leishmania amazonensis, L. chagasi, L. major,* and *L. mexicana* important intracellular protozoan parasites (Lemesre et al. 1997, Bourguignon et al. 1997, Holzmuller et al. 2002)
Giardia lamblia: NO generates peroxynitrite which kills protozoan trophozoites (Fernandez and Assreuy 1997)	Hepatoprotection: nitric oxide is involved in liver regeneration (Rai et al. 1998) and could suppress replication of hepatitis B virus in mice liver (Guidotti et al. 2000)
In hyperoxic pulmonary injury iNOS and NO had protective effects (Kobayashi et al. 2001)	Anti-Chagas disease: NO can kill *Trypanosoma cruzi* (Bourguignon et al. 1997)
Skin wound healing: NO helps to remove excessive production of superoxide anions contributing to cicatrisation (Shekhter et al. 2005, Kröncke and Suschek 2008)	Severe malaria: NO is essential to kill *P. falciparum* (Rockett et al. 1991, Balmer et al. 2000), but its massive release induced by free radicals and IgE worsen the infection
Penile erection, bladder control, lung vasodilation and gut peristalsis (Lamas et al. 1998, Napoli and Ignaro 2001)	Nitric oxide induces apoptosis on human cancer cells submitted to radiotherapy (Cook et al. 2004, Wang et al. 2003)

Indeed, nitrous acid can react with secondary amines resulting in the formation of highly carcinogenic nitrosamines, as represented below (Nappi and Vass 2002):

$R_2N\text{-}H$ (2^{nd} amine) + HNO_2 → $R_2\text{-}N\text{-}N{=}O$ (nitrosamine) + H_2O

Beyond those chemical reactions, NO can react with aminoacid residues triggering changes in the conformational structure of proteins, as noted below:

Protein-SH + RS-NO → Protein-S-NO + R-SH

NO AS THE MOLECULAR LINK BETWEEN INFECTION AND ATHEROSCLEROSIS

In the process of protein peroxidation, nitrosylation of tyrosine residues by peroxynitrite into lipoproteins can occur in the endothelial wall and inside the inflammatory infiltrates of coronary atheromas (Hogg et al. 1993, Berlett and Stadman 1997, Napoli and Ignaro 2001). In infections, the myeloperoxidase enzyme is overactivated, yielding reactive oxygen and nitrogen species. Infectious diseases agents such as *Chlamydia pneumoniae, Streptococcus* sp, *Porphyromonas gengivalis*, herpes simplex virus, coxsackie virus, and hepatitis virus can trigger immune-inflammatory responses in the endothelium as well as induce oxidative stress, increasing the risk of atherosclerosis (Lizard and Gambert 2001). The vasculitis induced by HIV infection (Aoun and Ramos 2000) could be the cause of the endothelial dysfunction and carotid artery stiffness found in HIV-positive children (Bonnet et al. 2004), and adults (Mercie et al. 2002). It has been suggested that during the course of chronic or multiple infection, myeloperoxidase also oxidizes NO_2^- yielding nitrogen dioxide ($NO_2^{\bullet}$) which nitrosylates proteins such as low-density lipoproteins (Epstein 2002, Pennathur et al. 2004). The "missing" link between infection, reactive species, and atherosclerosis can be worked out by reference to the following reaction (Byun et al. 1999):

$H_2O_2 + NO_2^- + H^+ \rightarrow NO_2^{\bullet} + HO^- + H_2O$

REACTIVE NITROGEN SPECIES IN DIABETES MELLITUS PATHOGENESIS

Experimental studies have suggested that the induction of iNOS expression and subsequent

Table 2. **Pathological aspects of nitric oxide, peroxynitrite and nitrogen reactive species**

- Acute renal ischemia: NO generated peroxynitrite that induced renal ischemia (Noiri et al. 2001)
- Air fine particulate pollution: decreases expired NO compromising lung function (Kim et al. 2003)
- Alzheimer disease (AD): amyloid- β-peptide generates peroxynitrite by NO mitochondrial releasing; peroxynitrite damage activated microglial cells in brain (Xie et al. 2002); peroxynitrite induced nitrosylation of tyrosine in brain of AD patients (Smith et al 1997); amyloid-β activated mitochondrial release of nitric oxide which in turns impaired mitochondrial ATP synthesis, triggering apoptosis of neurons (Keil et al. 2004)
- Atherosclerosis: decreased levels of NO can be found in plasma of atherosclerosis patients (Sözmen et al. 1999); insufficient interaction of NO with guanylate-cyclase, and consequently cGMP deficits, impaired endothelial vasorelaxion increasing atherogenesis (Napoli and Ignaro 2001)
- Autism patients present increased levels of red blood cell NO and plasma GPx (Sogut et al. 2003)
- Bacterial infections: NO increased *Escherichia coli* infection (Dyet and Moir 2006)
- Chronic obstructive pulmonary disease is related to increased nitrosative stress (Tsoumakidou et al. 2005)
- Cigarette smoking releases peroxynitrite (Yamaguchi et al. 2000)
- Colitis: NO induced damage to colon cells in colitis (Roediger 2002)
- Cystic fibrosis: NO deficiency impaired bronchial relaxation contributing to airway obstruction (Mhanna et al. 2001)
- Diabetes mellitus: mitochondrial dysfunction; neuropathy (Hoeldtke et al. 2002, Mastrocola et al. 2005, Obrosova et al. 2007)
- Eclampsia and intrauterine growth retardation pregnancies were both associated with enhanced levels of NO; lipid peroxidation was higher only in eclamptic patients (Pasaoglu et al. 2003)
- Endotoxin-induced hepatic damage: inhibition of nitric oxide synthesis caused DNA damage and impairment of hepatic microcirculation, aggravating hepatic damage (Takemura et al. 2000)
- Gastric cancer: higher NO, nitrate, and malondialdehyde levels were found in the plasma of gastric cancer patients; those biomarkers were associated with disease severity (Bakan et al. 2002)
- Hepatitis: peroxynitrite promoted nitrotyrosine formation increasing the severity of chronic viral hepatitis (Garcia-Monzon et al. 2000)
- Hemorrhagic hipovolemic shock: iNOS is activated in hemorrhagic shock yielding excessive amounts of NO which in turn is converted into peroxynitrite through superoxide generated by NADPH-oxidase (Szabó and Thiemermann 1994, Abdelrahman et al. 2005). NO is also involved in NFκB-induced TNF-α-induced damage after hemorrhagic shock (Altavilla et al. 2002) and triggers cyclooxygenase-2 expression and PGE_2 synthesis inducing multi-organ damage (Md et al. 2005)
- Hypertensive patients had lowered plasma concentrations of nitric oxide (Sözmen et al. 1998)
- Influenza virus infection: NO reacts with superoxide yielding peroxynitrite which potentially aggravates lethality by pneumonia (Akaike et al. 1996)
- Lung pathology: higher expression of iNOS, production of NO, and exhaled NO were associated with lung allergy and asthmatic inflammation (Koarai et al. 2002, Thomas et al. 2005)
- Methamphetamine induced formation of peroxynitrite and nytrotryrosine causing injury to dopaminergic neurons (Imam et al. 2001)
- Multiple sclerosis: an study suggests that excessive nitric oxide production could impair leucocyte function in MS patients (Mayer and Hermanova 1999)
- Oral cavity cancer: patients had increased erythrocyte levels of MDA and NO, and decreased content of antioxidant enzymes (SOD, CAT, GPx) (Beevi et al. 2004)

- *Plasmodium falciparum* malaria: NO has paradoxically effects on the pathophysiology of malaria. NO is essential to the destruction of parasites but its excessive generation through massive iNOS activation can worsen brain malaria (Angina and Abd-Allah 1999, Mazie and Idrissa-Boubou 1999, Lopansri et al. 2003)
- Sickle cell disease: available NO is consumed to yield peroxynitrite. The last could yield hydroxyl radicals and nitrogen dioxide ($NO_2^{\bullet}$), amplifying the nitrosative deterioration of membrane phospholipids in erythrocytes, damaging also aromatic amino acids by nitration, and contributing to lipoprotein oxidation (Aslan et al. 2000). Sickle cell anemia patients have leg ulceration, vascular painful injuries, and pulmonary hypertension which can be reversed by use of nitric oxide donors (Weiner et al. 2003, Kato et al. 2006, Machado et al. 2007)
- Skin: NO has paradoxically effects on skin, because it can protect it but also damage it through peroxynitrite-induced nitrosative reactions in thermal skin injury (Oliveira et al. 2004)
- Spinal cord injury is associated with NO, peroxynitrite and nitrotysine formation (Liu et al. 2000)

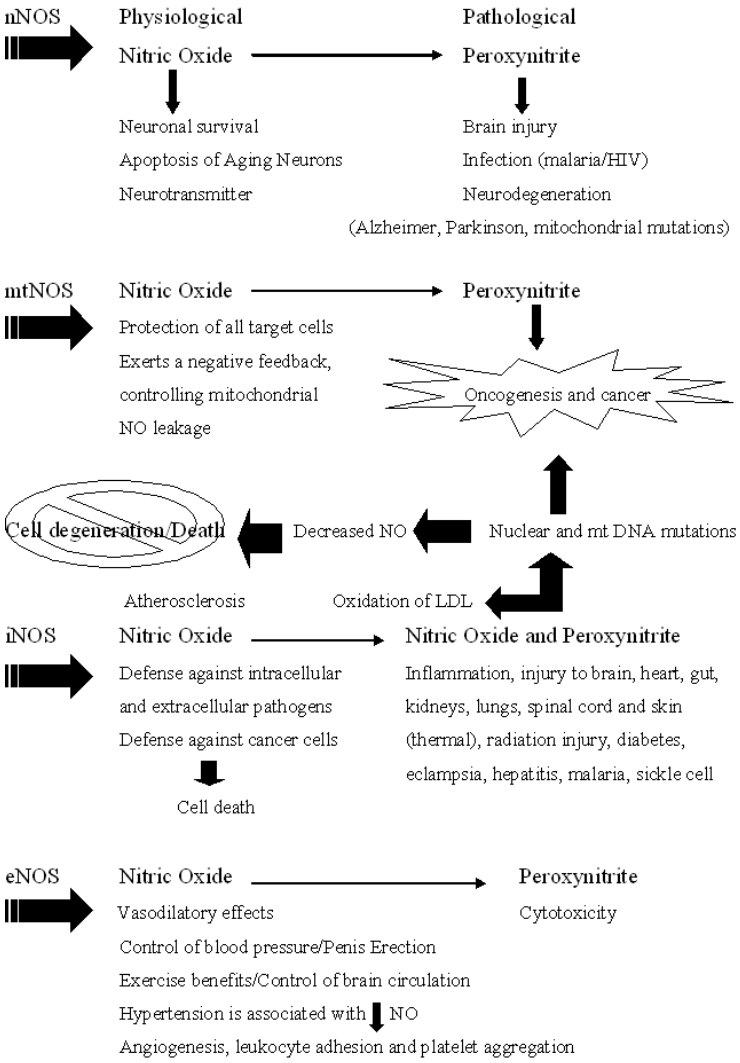

Fig. 1. **Conceptual framework for physiological and pathological roles of nitric oxide synthase isoforms, nitric oxide and peroxynitrite**

NO-ONOO• are associated with lesion severity in acute pancreatitis (Al-Mufti et al. 1998). It has been suggested that diabetic β-cell destruction is triggered by the cytotoxic effects of peroxynitrite (Lakey et al. 2001, Yu et al. 2002) and that this effect is, at least in part, mediated by mitochondrial dysfunction damage (Mastrocola et al. 2005). Endot helial impairment due to NO deficiency is a common feature of diabetic patients (Hogikyan et al. 1998). In this sense, endothelin-1, an NO antagonist, increases endothelial vascular resistance whereas its suppression restores NO bioavailability and vasodilatory effects among diabetic subjects (Mather et al. 2004). Insulin sensitivity is preserved in HIV patients with endothelial dysfunction, despite the toxic endothelial effects of indinavir (Shankar et al. 2006). In spontaneously hypertensive rats, insulin resistance was associated with the inhibition of NO synthesis by phosphatidilinositol-3-kinase (PI3K) suppression as well as increased endothelin-1 production through the mitogen-activated protein kinase (MAPK) pathway (Potenza et al. 2005).

Hoeldtke et al. (2002) have demonstrated that nitrosative stress depleted uric acid nerve stores leading to the functional degeneration of peripheral nerves. Decomposition of peroxynitrite ameliorated diabetic neuropathy in two animal models (Obrosova et al. 2005). It has been observed that blocking of peroxynitrite by Fe(III)tetrakis-2-(N-triethyleno glycol monomethyl ether) pyridyl porphyrin (FP15) reversed nitrosative stress-induced vasoconstriction as well as neuropathic damage in streptozotocin-diabetic rats (Obrosova et al. 2007).

Beyond its effects in diabetes, peroxynitrite is associated with toxic effects capable of inducing hemolysis of human erythrocytes (Kondo et al. 1997), killing motor neurons of amyotrophic lateral sclerosis patients (Estéves et al. 1999), and disturbing the surfactant function, all resulting in the induction of inflammatory reactions (Weinberger et al. 2001).

Recently, it has been found that insulin administration improves melatonin antioxidant activities in macrophages from alloxan-induced diabetic rats (França et al. 2009).

DO REACTIVE NITROGEN SPECIES WORSEN INFECTION?

During the course of an infection, the enhanced mitochondrial energy metabolism generates nitric oxide which, in turn, can help to destroy pathogenic bacteria, fungi and protozoa. The lack of nitric oxide synthesis from leucocyte phagocytes is associated with an increased risk of infection by *Porphyromonas gingivalis* (Gyurko et al. 2003), *Trypanosoma cruzi* (Hölscher et al. 1998), *Plasmodium falciparum* (Boutlis et al. 2003) and many other pathogens. Nonetheless, when the capacity of blood leucocytes is normal or increased, iNOS can generate significant amounts of nitric oxide that could inhibit the invasion of macrophages and fibroblasts by *Ricketsia prowasekii* (Turco et al. 1998). In a similar manner, NO generate peroxynitrite which is essencial for the destruction of *Candida albicans* (Vazquez-Torres et al. 1996) and the *Trypanosoma cruzi* infection is also controlled by a massive nitric oxide release from stimulated macrophages (Talvani et al. 2002). However, excessive amounts of nitric oxide and peroxynitrite aggravate infections. Astrocytes and glial cells activated by Gram-positive bacteria release excessive levels of nitric oxide contributing to neuronal damage (Kim and Täuber 1996). In relation to this, a study (Angina and Abd-Allah 1999), has reported that NO is associated with a worsening of malaria brain infection whereas other research (Lopansri et al. 2003) has observed that NO deficiency is associated with severe falciparum malaria, NO decreases the expression of intercellular and vascular cell adhesion molecules (ICAM-1 and VCAM-1) inhibiting *P. falciparum* adhesion to the endothelium of brain vessels, but the parasite induces the release of immunoglobulin E and subsequent NO overload which aggravates cerebral malaria (Mazie and Idrissa-Boubou 1999). A large number of nitrosative stress-induced pathologies are listed in Table 2. A conceptual model of nitric oxide and peroxynitrite in the protection of, or damage to, human tissues and cells is represented in Fig. 1.

CONCLUSION

Oxidative and nitrosative stresses are involved in a great number of pathophysiological states. Their adequate diagnosis and control by antioxidant dietary intake as well as a better lifestyle by diet and exercise could reduce the risk of diseases and even improve disease management and control.

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