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Review article

Nitric Oxide Functions; an Emphasis on its Diversity in Infectious Diseases

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Abstract

Nitric oxide is a short-lived mediator, which can be induced in a variety of cell types and produces many physiologic and metabolic changes in target cells. It is important in many biological functions and generated from L-arginine by the enzyme nitric oxide synthase. Nitric oxide conveys a variety of messages between cells, including signals for vasorelaxation, neurotransmission and cytotoxicity. In macrophages, nitric oxide synthase activity appears slowly after exposure of the cells to cytokines and bacterial products, is sustained, and functions independently of calcium and calmodulin. The cytokine- inducible nitric oxide synthase (iNOS) is activated by several immunological stimuli, leading to the production of large quantities of nitric oxide which can be cytotoxic. To date, there have been conflicting reports concerning the clinical significance of nitric oxide in infections. Some authors have proposed that nitric oxide contributes to the development of severe and complicated cases, while others have argued that nitric oxide has a protective role. The aim of this review is to evaluate the functions of nitric oxide production toward oxidative stress induced by infections or inflammations. It is indicated that NO is an important, but possibly not essential contributor in the control of acute phase of infections and it is only part of an immunopathological chain against pathogens. The anti-microbial function does not relate only to nitric oxide action or its related molecules, a combination of nitric oxide and immune factors is required to resolve pathogenic micro-organisms. Consequently, the NO theory in infectious diseases may lead to the novel ideas for therapy and prevention.

Keywords: Infection, Infectious disease, Nitric oxide, Nitric oxide synthase, Reactive nitrogen intermediate

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Introduction

The nitric oxide (NO) molecule consists of two atoms of oxygen and nitrogen, bound by a double bond. NO has a specific partial molecular polarity with a negative and positive charge on the oxygen and nitrogen atoms respectively (1). NO is a product of L-arginine conversion to L-citrulline by nitric oxide synthase (NOS) enzyme in the presence of nicotine amide adenine dinucleotide phosphate (NADPH) as a co-factor. NO is a reactive free radical, and in the presence of oxygen, is oxidised to a variety of nitrogen oxides (1). NO is known to react rapidly with oxyhemoglobin (*Oxy*-Hb) to give nitrate and *met*-Hb (2).

In addition, NO is a short-lived biological mediator produced by many cell types to induce many functions (3). It has recently been found to be a potent immuno-modulator, which has alternative roles during inflammation, infection and transplant rejection (4). Both oxygen and NO are vital for life processes, but too much of either can damage cells. It is suggested the attachment of NO to proteins enable them to activate gene(s) directly, but the body needs to keep NO in equilibrium by turning on and off expression of NOS gene(s) (5). NO also, has an extraordinary physiological role with an ability to diffuse freely through cell membranes to induce unrelated phenomena among different type of cells offering a new perspective on cell-cell communication (6). NO is produced in the neutrophils, lymphocytes and macrophages as part of the cytotoxic function of these cells (7). Macrophages, neutrophils, Kupffer cells and hepatocytes are stimulated to produce reactive nitrogen intermediate (RNI) *via* TNF-α and IFN-γ pathways (8).

NO acts as both a pro-inflammatory and an anti-inflammatory agent. The mechanisms that underline these effects remain poorly defined (9). Chronic inflammation is known to be associated with increased levels of both NO and reactive oxygen intermediate (ROI) including O_2 and H_2O_2 (10). It is now recognised that NO is not only involved in acute, but also, in chronic inflammation e.g. formation of a cellular granuloma (11). In some chronic inflammatory states, target cells may be exposed to high amounts of NO as much as 10^4 molecules NO per cell (10).

Although immunity to infections is complex and not properly understood, a number of different effector mechanisms in addition to NO have been implicated (12). It is suggested that a cascade of reactions leading to NO production are involved in infection processes (13). There are some contradictory reports about the role of NO and related molecules in infectious diseases. Some researchers propose NO is involved in the development of severe diseases, whereas, others argue a protective role for NO (14). Due to its contradictory actions, it is still an open question as to whether NO effects are protective or damaging (15).

We have previously published the detailed applications of detection assays for NO and its up/down stream molecules, NO-modulators, time courses and the associated changes in NO and its metabolite concentrations of host infected by some intracellular parasitic infections (16-19). These will provide a considerable help for this review to be discussed and clarified on NO functions with an emphasis on its diversity in infectious diseases.

Cytotoxicity of NO

Mononuclear cells and several other mammalian cells produce NO after stimulation with cytokines, bacterial endotoxin or antigens of infectious agents (11). Endothelial and Kupffer cells can be rich sources of cytotoxic levels of NO (20) and elevated levels of RNI and ROI can cause tissue damage (21). Peroxynitrite ($ONOO^-$) and hydroxyl radical ($OH^\bullet$) species oxidise lipids, thiol groups and damage cell membranes (22). In spite of its toxic role during the formation of $ONOO^-$ in phagocytosis and inflammation, NO has also, many non-toxic functions (23). Activated macrophages have an important role in the antimicrobial and antitumoural activity of NO (24). Generated NO has cytotoxic properties against tumour cells, intracellular bacteria, protozoa, extra-cellular fungi and helminths (25, 26).

Microbicidal and tumouricidal activities of NO

The antibacterial, antiviral and antifungal actions of NO *in vitro* in many culture media and *in vivo* in animal and human models are fully explained (27-29). NO exerts its protective function not only through direct antibacterial action, but also by preventing apoptosis and thereby contributing to antimicrobial defence during salmonellosis (30). Patients with brucellosis had significantly elevated serum levels of NO compared to healthy controls (31). In addition, NO is produced during *Pseudomonas aeruginosa* keratitis and may protect tissues from damage (32) or iNOS expressions were well correlated with *Helicobacter pylori* density, acute and chronic inflammation of gastric mucosa (33). In human immunodeficiency virus (HIV) negative and HIV co-infected tuberculosis (TB) patients, low levels of exhaled NO were observed which could be a risk factor in acquiring TB and the relative importance of NO in human TB (34). Moreover, as an antifungal, NO plays an important role in platelet-activating factor (PAF)-induced protection against systemic candidiasis caused by *Candida albicans* (35). However, there are some reports indicating no protective effects or unchanged NO levels during some infections e.g. chronic hepatitis C (36).

The possible key role of NO in the killing of infectious agents and cancer cells should be considered during control of infection and tumour cell growth (4). However, there are substantial literatures on NO suggested a potent antimicrobial role for NO. Both NO and NO_2 inhibit growth, respiration and active transport of fungi and bacteria (26). Some pathogens including *Herpes simplex, Cryptococcus neoformans* are reported to be inhibited by RNI (37), therefore, NO formation may be one of the principle mechanisms for decreasing infection (38).

Antiparasitic effects of NO, RNI and ROI

A role for antiparasitic effects of NO and RNI *in vivo* and *in vitro* have been demonstrated against a number of parasites including *Plasmodium spp., Leishmania spp., Toxoplasma gondeii, Schistosoma spp*. and *Trypanosoma brucei* (2, 15, 23, 37-41). Antiparasitic activity of NO has been illustrated with two most important malaria and leishmania parasites (42). NO reported to destroy some intracellular parasites such as *L. major* (43). Moreover, ROI produced by macrophages and granulocytes are responsible for phagocytosis of *Try. cruzi, Toxo. gondeii, Leishmania spp.* and *Plasmodium spp*. (23). There is evidence that the killing of intracellular parasites (*L. major, Toxo. gondeii*) and extracellular parasites (*Schistosoma spp*.) by activated macrophages through IFN-γ, correlates with the release of NO and RNI (23, 26). NO production has been shown to act directly as a leishmanicidal factor of parasitised macrophages. It seems that NO is not only necessary, but is also sufficient to account for the entire antiparasitic activity (42). The roles of NO and superoxide have been also described in the killing of enteropathogenic protozoa e.g. *Giardia lamblia* (44). The role of NO on human *mansoni* schistosomiasis points to a possible regulatory role of NO in the development of granulomas (11). Furthermore, the formation of NO, RNI and $ONOO^-$ has been reported for other parasites including *L. amazonensis* (45), *Opisthorchis viverrini* (46), *Clonorchis sinensis* (38) and *L. mexicana* (47). Increased NO synthesis might have a protective rather than pathological role in the majority of parasitic infections including malaria (39) and leishmania (19). Therefore, the involvement of NO and its up/downstream metabolites in parasitic infections is in the agenda and under debate; however it is required more investigations.

NO and C-reactive protein (CRP)

In addition to NO, CRP is a major acute phase protein present in normal serum, which increases significantly after most forms of tissue injuries and infections as a non-specific innate defence mechanism of the host. CRP as a protein is mainly regulated at the transcriptional level, induced by cytokines (48). It is a marker of inflammatory reactions and cytokine activation (49), which is

produced very early after infection (50). CRP is reported to be a critical element during a majority of infections (51). The data have been revealed a correlation of CRP and NO in some infections. This may clarify the co-involvement of CRP and NO as two major immune elements during infection (52); however, it is not justified, whether the CRP / NO production is beneficial or detrimental to the host. Not withstanding the conflicting publications, the role of CRP (53) and NO (54, 55) in the immune responses to infections remains uncertain. It is suggested that NO alone or in accompany with CRP and other chemokines is involved in protective or pathogenic responses of human infections (52).

Antimicrobial effects of NO by DNA damage

NO can directly attack many molecules including DNA, but the reaction rate is very slow. It also inactivates ribonucleotide reductase, a key enzyme in the biosynthesis of DNA (24). NO can inhibit several intracellular enzymes or change cellular gene transcription machinery e.g. inhibition of DNA binding to the transcription factor (56). NO has also been shown to cause G:C→A:T transitions, DNA strand breaks (57), induction of oxidative DNA damage in activated macrophages and inhibition of enzyme(s) involved in DNA repair (58). There are some studies indicating the effect of NO on DNA damage (59), activation of poly ADP-ribose polymerase (PARP) (60), inhibition or reduction of DNA repair enzymes in DNA synthesis (61), inhibition of DNA-methyl transferase (57), inactivation of ribonucleotide reductase (62) and promotion of nitrosative deamination of DNA bases (10). One of the DNA modifications induced by exposure to $ONOO^-$, NO and O_2 is the formation of 8-nitroguanine and 8-oxoguanine as well as DNA single strand breakage (63). Mutagenesis and carcinogenesis are promoted by alkylation of specific sites in DNA or bases (G to O6-methylguanine), or on the ring-nitrogen positions in A, G, C and T. DNA repair proteins are also inhibited by NO-derived agents such as N_2O_3 *in vitro* and *in vivo* (10).

Antimicrobial effects of NO on mitochondrial respiration

Another intracellular target for NO is mitochondria. It is known that activated macrophages inhibit mitochondrial respiration. NO changes the ion currents through the mitochondrial membrane leading to the release of Ca^{2+} into the cytosol (57). Two distinct effects of NO reported for mitochondria are: *i*) accelerated onset of swelling Ca^{2+} loaded transition, and *ii*) changing the permeability of mitochondria (64). It is suggested that mitochondria are inactivating the NO iron-dependent enzyme system, controlling mitochondrial respiration and chemical energy (65).

Antimicrobial effects of NO by apoptosis

NO may contribute to the development of apoptosis, an endogenous process of programmed cell death (24). NO appears to cause both apoptotic and carcinogenic effects. It may inhibit induction of the tumour-suppresser protein (P53) as a guardian of the genome (59). There are some studies reporting apoptosis and necrosis induced by NO, in human chondrocytes (65), rat neurones (66) and rat Islet cells (67). Pro-apoptotic effects are caused by high amounts of NO produced by inducible NOS (iNOS), but anti-apoptotic effects can occur due to the continuous activity of endothelial NOS (eNOS) (68). Consequently, NO causes apoptotic and necrotic cell death in susceptible cells, depending on cell type and the time of exposure (59).

Pathological effects of NO by neurotransmisson and vascular tone in host

One of the important features described for NO is neurotransmission. In both the brain and peripheral nervous system, NO is implicated in neurotoxicity associated with stroke and neurodegenerative diseases (4). NO is a neuromodulator in its own right and may impair consciousness by increasing acetylcholine release and inhibiting N-methyl-D-aspartate (NMDA) activity in the brain. The most important type of NOS enzyme to be involved in neurotransmisson is suggested as

neuronal form of NOS (nNOS) (69). NO and O_2 in endothelium modulates vascular tone as a vasodilator substance (14). In addition, NO modulates basal coronary artery tone and it is responsible for the flow-mediated vasodilatation (70).

Other functions of NO in infections

NO may reacts with proteins and nucleic acids by binding to haem groups, guanylate cyclise (GC), Hb and cytochrome-C oxidase. Theoretically, NO may reacts with nucleophylic centres like sulphur, nitrogen, oxygen and aromatic carbons. It seems SH groups are prime targets for NO [30]. NO and O_2 rapidly react to produce $ONOO^-$, which is a potent oxidant of proteins, lipids and DNA (38). There are some examples of damage of cell membrane integrity, apoptosis, changes in cell cycle and DNA strand breaks, which were induced by NO and its metabolites (71).

Conclusive remarks

Taken together, the data provided by researchers, highlight the fact that NO and / or its related molecules have many functions and are involved in a large number of inflammations, infectious diseases, and biomedical concepts, but the involvement is not independent of other immune events. It is indicated that NO is an important, but possibly not essential contributor in the control of acute phase of infection. Although, the protective immune responses against micro-organisms is multifactorial and the final effector molecules that mediate organism death are not known, NOS, NO and RNI have been significantly implicated (72-74). It is concluded that NO is only part of an immunopathological chain against infection and the antimicrobial function does not relate only to NO action, so, a combination of NO and other immune factors is required to resolve pathogens. Therefore, the involvement of NO and its up / downstream molecules as an immuno-protective target in infections is highly under debate; and it is required more investigations to be resolved (75-77).

An association of some key cytokines appears to be essential for NOS gene regulation. Perhaps, NO comes from several cellular sources, further study in defining these sources will be important for the understanding of cell-mediated defence mechanism(s) in infectious diseases. However, the involvement of NO in infected host is conflicting, the complex relationship between symptoms, genetic polymorphisms and NO production in populations require more studies to address their immunomodulatory roles in viral, bacterial, parasitic and fungal infections (78, 79).

Although, the knowledge about cytotoxic effects of NO is steadily increasing, we are still at the beginning of understanding as to how, why, when and where cells are affected by NO. Consequently, the NO theory in infectious diseases may lead to the novel ideas for therapy and prevention (80).

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