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Roles of Nitric Oxide in Surgical Infection and Sepsis

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Abstract. Recent advances in nitric oxide (NO) research have begun to elucidate the roles of NO in sepsis and infection. Although adequate levels of NO production are necessary to preserve perfusion and carry out cytoprotective functions in sepsis, overproduction appears to contribute to hemodynamic instability and tissue damage. These observations have led to the development of strategies to inhibit NO synthesis or scavenge excess NO in patients with septic shock. Local expression of the inducible NO synthase also has antimicrobial functions. The combination of NO with superoxide forms peroxynitrite which participates in bacterial killing in the peritoneal cavity. The capacity of red blood cells and hemoglobin to remove NO most likely accounts for the adjuvant effect of blood in peritonitis.

This review will summarize the pathobiology of NO in surgical sepsis and infection.

Often in biology the most complex processes are governed by the simplest of mediators. Such is the case for sepsis, where bacterial endotoxin (lipopolysaccharide; LPS), cytokines, and other small biologically active molecules initiate and propagate the cascade of events that culminate in unrelenting hypotension, multiple organ dysfunction, and frequently death. Nitric oxide (NO), one of the smallest synthetic products of mammalian cells, has leaped forward as an important contributor to the physiology and pathophysiology of sepsis. This review explores the diverse roles this mediator plays in clinical conditions familiar to surgeons: surgical infections and sepsis.

Biochemistry of Nitric Oxide

Furchgott and Zawadzki first made the observation in 1980 that endothelium was required for vascular smooth muscle relaxation in response to acetylcholine [1]. Seven years later Palmer et al. [2] and Ignarro et al. [3] identified NO as this endothelial-derived relaxing factor. Since then, NO has proved to be one of nature's most utilized and interesting molecules, with involvement in such diverse physiologic processes as neurotransmission, vascular tone, sphincter relaxation, microbial killing, and penile erection.

Nitric oxide is a relatively unstable, uncharged free radical. Its small size, absence of electrical charge, and lipophilic nature allow it readily to diffuse across cell membranes; although its short half-life (seconds) in aqueous, oxygenated solutions limits its effects to the local environment. Either NO or its reaction products target an abundance of molecular targets, including heavy metals, other radicals, and protein thiol groups. Through these interactions, NO modifies protein function and leads to or protects from cellular damage. NO oxidizes rapidly in the presence of oxygen or hemoglobin to form the measurable end-products nitrite and nitrate.

Nitric oxide is synthesized from the amino acid L-arginine via a family of enzymes termed nitric oxide synthases (NOS). Named for the cell type where they were first discovered, there are now three known isoforms of NOS—neuronal (nNOS, NOS1), inducible (iNOS, NOS2), and endothelial (ecNOS, NOS3)—each encoded by different genes (Table 1). Further study revealed that expression of all three isoforms is not limited to a single cell type. Two of these forms, endothelial and neuronal NOS, are constitutively expressed under normal conditions and are collectively called cNOS (reviewed in [4]).

All NOS enzymes catalyze the five-electron oxidation of the guanidino nitrogen of L-arginine to citrulline (Fig. 1), possess a protoporphyrin IX heme group, exist as homodimers, and are active only in the presence of cofactors nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and tetrahydrobiopterin (H_4B).

iNOS differs from the cNOS isoforms in both regulation of activity and expression. The cNOS enzymes are typically always present in cells, and their activity is regulated by intracellular calcium levels through a calcium/calmodulin-dependent mechanism. The binding of acetylcholine, bradykinin, histamine, and other vasoactive substances to endothelial cells results in a receptor-mediated calcium influx. Calcium binds the cytosolic calcium-binding protein calmodulin, which transiently binds cNOS, increasing NO production. NO then diffuses across cell membranes into adjacent smooth muscle cells, where it activates guanylate cyclase. Increased production of cyclic guanosine monophosphate (cGMP) activates cGMP-dependent protein kinases, ultimately leading to vasorelaxation. As intracellular calcium levels decrease, the stimulus for NO production is eliminated. In a similar manner, neurons expressing nNOS respond to excitatory amino acid stimulation by increasing NO production and increasing cGMP levels.

iNOS protein, as its name implies, is typically not present in

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Table 1. Nitric oxide synthase isoforms.

NOS isoform	Alternate name	NOS expression	NO production	Function/actions
Type I	Neuronal NOS	Mostly constitutive	Small, intermittent	Neurotransmission Cell signaling Neurotoxicity
Type II	Inducible NOS	Usually requires induction	Large, sustained	Cytoprotection Cytotoxicity Antimicrobial Immunoregulation
Type III	Endothelial NOS	Constitutive	Small, intermittent	Cell signaling Vasomotor tone regulation

NOS: nitric oxide synthase.

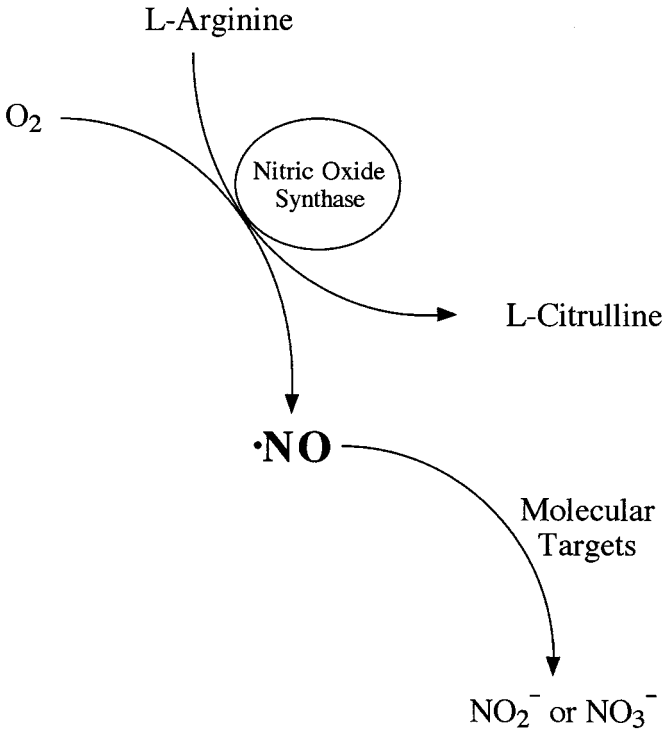

Fig. 1. Reaction of nitric oxide synthase (NOS). In the presence of molecular oxygen, NOS converts L-arginine to L-citrulline, creating nitric oxide (NO). NO reacts with a multitude of targets before oxidizing to the stable end-products nitrite (NO_3^-) and nitrate (NO_2^-).

unstimulated mammalian cells but is synthesized *de novo* in response to stimuli such as endotoxin or inflammatory cytokines such as tumor necrosis factor-α (TNFα), interleukin-1 (IL-1), and interferon-γ (IFNγ) [5, 6]. Hence there is a necessary time delay between stimulation and NO formation. Once formed, iNOS produces much higher quantities of NO and is maintained tonically active by a permanently bound calmodulin found in each subunit of the iNOS homodimer [7]. iNOS-mediated NO production is limited only by the concentration of enzyme and the availability of substrate and cofactors.

The potential for NO to participate in myriad physiologic processes is apparent from the study of its chemistry and regulation. The constitutive isoforms of NOS, with their tightly controlled, intermittent, low-level NO production, combined with the diffusibility of NO make for an ideal autocrine and paracrine messenger. Conversely, the inducible NOS isoform, with its ability to produce larger quantities of NO, allows the body to unleash the reactivity of NO in a variety of responses.

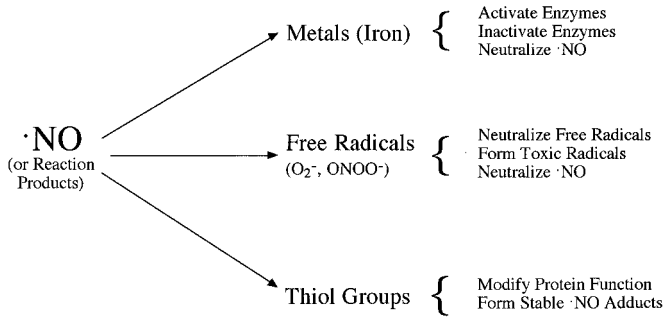

Fig. 2. Molecular targets of NO. NO reacts with several intracellular species, mediating cell signaling and protective and toxic effects.

Molecular Targets

Nitric oxide participates in cellular metabolism, signal transduction, and cellular protection and toxicity by interacting with a variety of cellular components including heavy metals, reactive oxygen species, and biologic molecules such as thiol groups (Fig. 2). One of the most biologically significant aspects of NO is its ability to react with intracellular metals and metal-containing proteins [8]. A prime example is the interaction with the iron heme moiety of guanylate cyclase. In that reaction, NO reacts with heme to liberate a transaxial ligand (histidine), resulting in enzyme activation and cGMP synthesis. In contrast, other heme proteins (e.g., catalase [9, 10] and cytochrome P450 [11]) are inhibited by NO. There is evidence to suggest that NO interacts with the heme prosthetic group of cyclooxygenase to either activate or inhibit the enzyme [12–14]. NO rapidly reacts with heme groups in hemoglobin and myoglobin to form iron nitrosyl hemoprotein, which may attenuate NO-mediated biologic functions [15, 16], as hemoproteins such as hemoglobin and myoglobin are the most powerful biologic scavengers for NO. NO can even regulate its own biosynthesis by reacting with the heme iron in both cNOS and iNOS [17–19]. This feedback inhibition may serve to control neurotransmitter release, endothelium-dependent relaxation, and NO-mediated cytotoxicity.

Nitric oxide also mediates cellular events by reacting with enzymes containing nonheme iron [20]. Such enzymes include mitochondrial *cis*-aconitase [21, 22], complexes I and II of the mitochondrial electron transfer chain [23–25], and ribonucleotide reductase [26, 27]. Through these interactions NO may inhibit cellular adenosine triphosphate (ATP) and DNA synthesis.

S-Nitrosylation of intracellular nucleophilic thiol groups by NO, or more likely NO products (e.g., nitrosonium, NO^+), activated by reaction with heavy metals or other radicals [8, 28] represents a newly discovered cell signaling mechanism and can regulate protein function in several ways (reviewed in [29]). Activation of calcium-dependent potassium channels in vascular smooth muscle

is mediated by a conformational change following *S*-nitrosylation [30]. The cytosolic glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is inhibited by nitrosylation of thiol groups located within the active site [31]. *S*-Nitrosothiols may also serve as a reservoir for NO [8, 32–35]. Jia et al. [36] suggested that *S*-nitrosylated hemoglobin functions as a NO carrier, contributing to both efficient oxygen delivery and distant vasomotor control.

As previously stated, NO reacts with oxygen radical species to yield a multitude of secondary products, some of which are more reactive and toxic than NO [37]. In many pathologic conditions, such as inflammation and endotoxemia, NO and superoxide anion (O_2^-) are produced simultaneously. The reaction of NO with O_2^- to form peroxynitrite ($ONOO^-$), a much stronger oxidant than either NO or O_2^-, has clear toxicologic significance [38, 39]. NO and O_2^- form $ONOO^-$ at near diffusion-controlled rates ($K = 6.7 \times 10^9\ M^{-1}\ sec^{-1}$) [40]. This reaction is fast enough to compete with superoxide dismutase (SOD) when elevated levels of NO are present. Protonation of $ONOO^-$ yields peroxynitrous acid (ONOOH, pKa = 6.8) [41], which decomposes to nitrogen dioxide ($^{\cdot}NO_2$) and an oxidant with hydroxyl radical-like ($^{\cdot}OH$) activity [38].

Cytoprotection of Nitric Oxide

The cytotoxic potential of NO—through inactivation of metabolic pathways, damage to cell structures and proteins, DNA mutation, and alterations of gene expression—is apparent. Alternatively, NO formation may serve to protect cells from reactive oxygen intermediates' (ROIs) cytotoxicity. Consumption of O_2^- by NO with ultimate degradation to nitrate could prevent accumulation of hydrogen peroxide (H_2O_2) and formation of hydroxyl radical ($^{\cdot}OH$) via the Fenton reaction [38]. NO can scavenge hydroxyl radicals [42] and inhibit radical-induced chain propagation during lipid peroxidation [43]. The delayed production of high levels of NO after bacterial infection may serve to neutralize the burst of ROIs produced more proximally in the inflammatory response, thereby preventing host cell injury.

Nitric oxide may be protective, as nonspecific inhibition of NOS following endotoxin or bacterial challenge increases hepatic damage [44–47], intestinal injury and splanchnic permeability [48], pulmonary hypertension [49], lung neutrophil infiltration [50], and microvascular permeability [51]. It decreases renal [52] and mesenteric and carotid blood flow [53] and causes myocardial ischemia [54]. The mechanism of this protective effect is unclear, but the observations that NO reduces O_2^- release [47] and oxygen radical scavengers prevent NOS inhibitor-associated liver injury [44, 45] suggest that NO scavenges toxic oxygen radicals. More recent evidence indicates that NO is a potent inhibitor of apoptosis in many cell types [55–57].

Nitric oxide may prevent endothelial damage by modulating platelet aggregation [58–60] and leukocyte adherence perhaps by altering the expression of adhesion molecules such as CD11/CD18 [61], selectins [62], and the intercellular adhesion molecule (ICAM) [63] or through the consumption of O_2^- [64]. Vasodilation due to increased NO synthesis would help to maintain tissue perfusion. Clearly this vasodilatory response could be detrimental if exaggerated. The role of NO in the unrelenting hypotension of septic shock is discussed below.

Cytotoxicity of Nitric Oxide

Although enzyme inhibition or toxic radical formation may explain NO-mediated control of target cell growth, whether they be microorganisms, tumor cells, or invading lymphocytes, the nonselective action of NO overproduction also undoubtedly explains some of its local cytotoxicity. Peroxynitrite inflicts cellular damage through lipid peroxidation [65], oxidation of sulfhydryl groups [41], nitration of tyrosine residues [28], and oxidation of cellular antioxidants [66, 67]. It has also been implicated in inactivation of the manganese/iron SOD [28]; it also may explain NO-mediated inactivation of aconitase [68, 69] and the mitochondrial electron transport chain [70]. NO or other reactive nitrogen intermediates can induce mutagenesis by deaminating purine and pyrimidine DNA bases or inducing DNA strand breaks [71–74]. Furthermore, by inducing DNA strand breaks, NO (or more likely peroxynitrite) activates the nuclear repair enzyme poly(ADP-ribose)polymerase (PARP). PARP activation results in a futile energy-consuming cycle, depleting intracellular NAD^+, thereby inhibiting glycolysis and ATP synthesis [75–77]. Szabó et al. have suggested that $ONOO^-$ activation of PARP may explain the energetic depletion of vascular smooth muscle cells and circulatory failure seen during sepsis [78, 79].

Lastly, in contrast to observations that NO blocks apoptosis, others have found that NO induces programmed cell death. Postulated mechanisms include *p53* induction or oxidant DNA damage [80–82].

Role of NO in Inflammation and Infection

Through tissue damage, NO or $ONOO^-$ promotes inflammatory changes, but there is also evidence that NO may regulate and promote inflammatory cytokine synthesis independent of tissue injury. NO enhances TNF and IL-8 release [83, 84] and activates cyclooxygenase, increasing formation of proinflammatory prostaglandins [13]. Conversely, NO may also play an antiinflammation role. NO reduces neutrophil oxidant production by inactivating neutrophil NADPH oxidase [85, 86], and it appears to downregulate cytokine production, as NOS inhibition increases IL-6 and TNF release and increases mortality after endotoxin challenge [87, 88]. Pretreatment with small doses of LPS protects animals from a second lethal endotoxin challenge, but pretreatment with the nonspecific NOS inhibitor L-N^G-methyl-L-arginine (L-NMA) abolished this protection, suggesting that NO upregulates immunity [89].

Nitric oxide appears to be part of the adaptive response to infection. It exerts antimicrobial activity against an expanding list of bacteria, parasites, fungi, and viruses (reviewed in [90]) [90–93]. NO is an important part of the macrophage arsenal, as inhibition of NO synthesis diminishes both microbicidal and tumoricidal activity [23]. Intracellular parasites are particularly sensitive to NO [94–96].

Several authors have postulated potential mechanisms for NO-mediated microbial killing. NO is the major molecule responsible for cytotoxicity of *Entamoeba histolytica* and may function via a superoxide-associated mechanism [97]. Host resistance to *Legionella pneumophila* may relate to IFNγ-mediated iNOS induction [98]. $ONOO^-$, not NO, may be the functional bactericidal agent [99] that kills *Escherichia coli* in concentrations achievable by activated macrophages [39]. Rubbo et al. further found that

peroxynitrite inactivates succinate dehydrogenase and NADH-fumarate reductase (via interaction with critical sulfhydryl residues), thereby inhibiting cellular respiration in *Trypanosoma cruzi* epimastigotes [100].

NO in Bacterial Peritonitis

Although the surgical world has long recognized the adjunctive effect of erythrocytes (RBCs) and hemoglobin on mortality in experimental bacterial peritonitis, the mechanisms for this observation were not clear for years [101, 102]. Erythrocytes have several antioxidant systems, including SOD, catalase, glutathione, glutathione reductase, and heme. RBCs can inhibit the oxidative bactericidal mechanism in superoxide-generating systems [103, 104]. Erythrophagocytosis can inhibit macrophage antibacterial function and oxidative bactericidal mechanisms [105, 106]. RBCs and hemoglobin have also been implicated in NO scavenging in vitro and in vivo [15, 107]. All of these points suggest that erythrocytes increase mortality associated with peritonitis by scavenging both reactive oxygen intermediates (ROIs) and NO, thereby quenching the microbicidal properties of these species.

Our group recently confirmed this hypothesis [108]. Peritoneal injection of live bacteria (*E. coli*) resulted in high levels of O_2^- and NO production. iNOS was strongly expressed in the leukocytes entering the peritoneal cavity in response to this infection. Administration of either RBCs or a combination of SOD, catalase, and L-NMA with a sublethal dose of live bacteria significantly increased intraperitoneal bacterial counts and mortality while reducing NO, O_2^-, and $ONOO^-$ formation. Thus although RBCs may protect cells and tissues from injury, peritoneal contamination protects bacteria from the oxidative bactericidal activity of the host defense system by scavenging O_2^- and NO in the peritoneal cavity (Fig. 3).

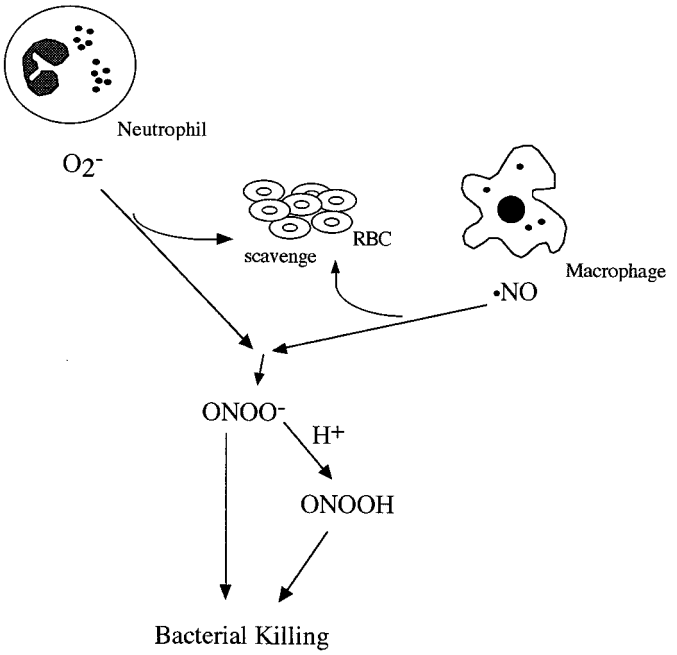

Fig. 3. Effect of erythrocytes (RBCs) on toxic radical formation. The RBCs scavenge cytotoxic oxygen and nitrogen radicals produced as part of the inflammatory response to intraperitoneal sepsis, reducing bacterial killing.

NO in Septic Shock

It is now clear that NO contributes to the pathophysiology of sepsis. NO synthesis is enhanced after exposure to endotoxin [109, 110] and the sepsis-associated cytokines IL-1β, TNFα, and IFNγ [5, 6]. Also, several investigators have demonstrated increased production of NO degradation products in patients with sepsis [111–114] and following cytokine immunotherapy for cancer [115, 116].

Role of NO in Hemodynamic Changes in Sepsis

A cardinal feature of septic shock is profound hypotension poorly responsive to fluid resuscitation and vasopressor therapy. NO overproduction may be an important contributor to this hypotension, as NOS inhibition increases blood pressure in animals challenged with endotoxin [117–120], TNF [121], IL-1 [122], and IL-2 [123] (reviewed in [124]). Although the nonselective NOS inhibitor L-NMA increases blood pressure in normal animals as well, the hypertensive effect is much greater in septic animals. Loss of vascular reactivity after endotoxin exposure also involves NO [125–128], as NOS inhibition improves the hyporeactive response to vasopressors seen with sepsis [129–131].

One potential discrepancy when attributing the hypotension of sepsis to increased NO production is the observation that induction of iNOS requires a 3- to 4-hour time delay before appreciable increases in NO production [132]. Some investigators have addressed this issue by noting that several septic mediators, although inducing formation of iNOS, also up-regulate constitutive NO synthesis [110, 124, 133]. Hence the hypotension noted shortly after endotoxin or TNF exposure is probably due to increased cNOS activity. Mechanisms of cNOS activation are unclear but may relate to LPS-induced release of a variety of mediators (bradykinin, histamine) that increase NO release from the endothelium (reviewed in [132]).

Nitric oxide is implicated in the cardiac dysfunction seen with septic shock. Inflammatory cytokines cause dose-dependent negative inotropic effects that are blocked by L-NMA treatment. The ameliorating effect of L-NMA can be reversed by adding substrate L-arginine [133, 134]. Thus NO not only may cause excessive vasodilation, it may contribute to decreased cardiac contractility.

Therapeutic Interventions

Therapeutic agents targeting individual mediators in the septic cascade have largely failed to show significant benefit in terms of mortality [135]. Most recently, several researchers have explored NO as a potential target for therapeutic manipulation (reviewed in [136]). As previously mentioned, NOS inhibitors cause an immediate rise in blood pressure and vascular resistance. However, administration of excessive doses of NOS inhibitors that are not selective for the inducible isoforms of NOS also cause concomitant inhibition of ecNOS and may increase the incidence of organ ischemia, microvascular thrombosis, and mortality [45, 48, 137]. A fall in cardiac output below normal levels has been reported in septic dogs and pigs treated with NOS inhibitors [49, 117, 138] but may relate to dosing and nonspecific NOS inhibition.

Survival studies using NOS inhibitors have not identified a clear effect. NOS inhibition increases mortality in several septic animal models [50, 87, 139–141]. Evans et al. concluded that NOS inhibition has no effect on survival [142], whereas, others have suggested that NOS inhibition improves survival [143, 144].

Maintenance of vascular perfusion by cNOS appears to play a protective role against acute shock and could explain the deleterious actions of NOS inhibition administered early in shock models. Early administration of nonspecific NOS inhibitors L-NAME or L-NMA results in widespread organ damage, whereas late administration after endotoxin reduces toxicity. This finding suggests that initial suppression of cNOS following LPS challenge aggravates injury, whereas delayed inhibition of iNOS provides protection against subsequent damage [137, 145–148].

Noting the conflicting results of inhibitor studies, Nava et al. [144] suggested that high-dose NOS inhibition could impair both constitutive and inducible NOS, effectively removing all NO-dependent vasodilatory tone. This reaction in the presence of circulating sepsis-induced vasoconstrictors, may lead to fatal end-organ damage. Nava et al. found a dose-dependent effect of L-NMA on blood pressure response. High doses (300 mg/kg) initially increased blood pressure but eventually caused a precipitous decrease in blood pressure and accelerated death, whereas a moderate L-NMA dose (30 mg/kg) or inhibition of iNOS induction with dexamethasone significantly attenuated the hypotensive response to endotoxin and improved survival. Additionally, infusion of an NO donor, *S*-nitroso-*N*-acetylpenicillamine (SNAP), reversed the deleterious effects on blood pressure and improved survival, suggesting that constitutive NO production contributes to blood pressure maintenance, whereas the inducible NOS is responsible for the hypotension and tissue damage of endotoxic shock [149].

Consistent with these observations, more specific iNOS inhibition has shown promising therapeutic potential. Aminoguanidine, a relatively selective iNOS inhibitor, decreases bacterial translocation after high-dose endotoxin challenge perhaps by reducing intestinal damage [150]; and it attenuates the delayed circulatory failure and mortality in endotoxemia models [151]. *S*-Methylisothiourea sulfate, a potent and relatively selective iNOS inhibitor, reverses hypotension and vasoconstrictor hyporeactivity caused by endotoxin while attenuating liver and renal injury and improving survival [152]. iNOS-specific NOS inhibition might reduce the circulatory failure and tissue toxicity associated with excessive NO production without the consequences of cNOS inhibition.

The successful administration of NOS inhibitors in septic patients has already been reported [153–156]. Petros et al., in the first human trial, treated two patients with septic hypotension unresponsive to high-dose norepinephrine with L-NMA and L-NAME (in one patient) and noted rapid increases in blood pressure and vascular resistance with clinical improvement in both patients, one of whom went on to survive [153]. This group then performed a randomized, double-blind trial where 12 patients received L-NMA bolus injections followed by constant infusion. Injections increased systolic and diastolic blood pressure and systemic and pulmonic vascular resistance while decreasing hyperdynamic cardiac output toward normal levels. They concluded that NO overproduction contributes to septic vasodilation, and that inhibition may be therapeutic [156].

Generalizations from animal studies to potential human interventions are limited for several reasons. Important species differences exist. NOS inhibitor doses used in animal studies have generally been much larger than those used in humans. The proper dose of NOS inhibitor is still not established, nor is the appropriate timing for NOS inhibition. The nonspecific nature of clinically available inhibitors also limits their widespread application.

The inability to create a highly selective iNOS inhibitor has led researchers to explore other means of elucidating the relative contributions of iNOS and cNOS to inflammation. The embryonic disruption of the iNOS gene in mice has led to mice that do not express iNOS protein (iNOS knockouts). They display similar growth rates, behavior, and reproduction as control mice. They do not, however, show increased nitrite and nitrate synthesis after endotoxin stimulation [157–159]. Survival studies using high doses of endotoxin have revealed conflicting results in these mice. Laubach et al. found no significant survival differences between iNOS knockout and wild-type mice [159]. MacMicking et al. made the contrasting observation that anesthetized knockout mice were significantly protected from moderate-dose endotoxin-induced hypotension and death; but only at high doses of endotoxin (30 mg/kg) were unanesthetized iNOS knockout mice protected [157]. In contrast, Wei et al. [158] found that iNOS knockout mice were indeed protected from intraperitoneal endotoxin challenge. The discrepancies in these early observations have not been adequately explained. iNOS may play a more subtle role than survival from overwhelming doses of endotoxin, or other iNOS-independent death mechanisms may exist.

Other researchers have explored the role of inhaled NO in the treatment of pulmonary disease. Unlike systemic vasodilators but like prostacyclin, inhaled NO reduces pulmonary artery pressure without inducing systemic hypotension. The absence of systemic hemodynamic effects probably reflects the rapid binding and inactivation of NO by hemoglobin. In experimental models of sepsis and oxidant lung injury, pulmonary hypertension, gas exchange, and ventilation-perfusion mismatch are all improved with NO inhalation [160–165]. The mechanism probably involves selected increased perfusion of ventilated alveoli or antiinflammatory effects of NO [166]. Clinical trials of inhaled NO have yielded promising results in patients with severe adult respiratory distress syndrome (ARDS) and pulmonary hypertension [167–169]. Controlled trials of inhaled NO in septic patients are awaiting completion.

Other areas currently being explored to assess the contributions of NO to sepsis include the use of NO scavengers, substrate and cofactor manipulations, the use of site-specific NO donors, and the application of gene transfer technology [170]. The use of NO scavengers that remove only the "excess" NO regardless of source present an attractive approach, especially because it is probable that some iNOS activity is beneficial. Kazmierski et al. have identified iron chelators that scavenge excess NO and protect against a lethal murine model of endotoxic shock [171]. Other approaches are less specific. For example, inhibition of synthesis of the NOS cofactor tetrahydrobiopterin reduces NO production and partially ameliorates endotoxin-induced hypotension in rats [172]. Methylene blue, which does not affect NOS but does inhibit guanylate cyclase, improves blood pressure and cardiac function in septic patients [173, 174].

Methods to support NO availability suggest that a relative NO deficiency may exist at certain intervals or specific anatomic sites during sepsis. For example, dietary arginine supplementation

improves survival in animal models of sepsis perhaps by preventing intestinal damage and bacterial translocation [175]. Also, NO donors attenuate endotoxin-induced intestinal damage and hemodynamic abnormalities, suggesting that an appropriate level of NO availability may be beneficial during sepsis [176, 177].

Conclusions

A review of the literature exploring the role of NO in sepsis reveals several paradoxes. NO appears to be both cytoprotective and cytotoxic. NO appears to block and induce inflammation. NOS inhibitors improve hemodynamics and reduce organ injury, although other researchers have insisted that NOS inhibitors are detrimental, reducing cardiac performance, worsening oxygenation, and hastening demise.

One answer to the NO paradox involves the use of multiple experimental models. High-dose NOS inhibition, with impairment of both the cNOS and iNOS isoforms, routinely results in tissue damage and deleterious outcomes, whereas lower-dose and more specific iNOS inhibition tends to have protective effects. As we take advantage of the pharmacologic developments of more selective inhibitors of iNOS, as we become more familiar with dosage and timing variables, and as other tools (e.g., genetically manipulated mice) become available, we may further define the role of NO and the NO synthase isoforms and thus effect some therapeutic benefit.

On the other hand, it is not surprising that a molecule with such diverse chemical capabilities can have such divergent effects. At physiologic concentration, NO is protective; it provides necessary vasodilation, inhibits platelet and leukocyte aggregation, and participates in both protein modification and local and perhaps distant cell signaling via nitrosothiol formation. But during pathologic conditions, overexpression exposes the environment to its toxic potential with toxic radical formation, protein inhibition, and DNA damage. Complete inhibition may not be desirable. Inadequate or excessive NO production is ultimately harmful to the host, resulting in hypercoagulability, altered perfusion, uncontrolled infection, tissue injury, and hypotensive shock. Appropriate amounts of NO as a part of the inflammatory response result in host survival. The effect of NO therefore depends on the rate and duration of NO formation, the presence of reactive substrates and the cell-specific response to the presence of NO.

Résumé

Certains progrès récents dans la recherche du monoxyde d'azote (MO) semblent indiquer le rôle que ce composé joue dans l'infection et le sepsis. Bien qu'un certain niveau de production de MO soit nécessaire pour conserver la perfusion et accomplir les fonctions de cytoprotection dans le sepsis, la surproduction de MO apparait comme un facteur d'instabilité hémodynamique et de lésions tissulaires. Ces observations ont amené à développer des stratégies thérapeutiques pour inhiber la synthèse de MO ou éliminer le MO en excès chez le patient en choc septique. L'expression locale de l'enzyme MO synthase inductible a également des répercussions sur les fonctions antimicrobiennes. La combinaison de MO avec la superoxyde est responsable de la formation de peroxynitrite qui participe à la mort bactérienne dans la cavité péritonéale. La capacité des globules rouges et de l'hémoglobine à extraire le MO rend compte sans doute de l'effet adjuvant du sang dans la cavité péritonéale. Dans cette revue, on résume la pathobiologie de le MO dans le sepsis en chirurgie et l'infection.

Resumen

Los recientes avances en la investigación del óxido nítrico (ON) aportan claridad sobre su papel en la sepsis y la infección. Aunque en la sepsis se requieren niveles adecuados de producción de ON para preservar la perfusión y cumplir las funciones citoprotectoras, la producción exagerada parece contribuir a la inestabilidad hemodinámica y al daño celular. Tales observaciones han dado lugar al desarrollo de estrategias tendientes a inhibir la síntesis de ON y la remoción de su exceso en pacientes en shock séptico. La expresión local de la sintasa inducible del ON también posee funciones antimicrobianas. La combinación de ON con superóxido forma peroximetrito, el cual participa en la eliminación bacteriana de la cavidad peritoneal. Muy probablemente la capacidad de los eritocitos y de la hemoglobina para remover ON explica el efecto coadyuvante de la sangre en la peritonitis. En el presente artículo se resume la patobiología del ON en las sepsis y la infección quirúrgica.

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