

Nitric oxide – a pro-inflammatory and anti-inflammatory mediator

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Abstract

NO is an ubiquitous mediator in cardiovascular, nervous and immune systems. Here we briefly review the role of NO in chronic inflammation and septic shock. In particular, we present the evidence that the double nature of NO resulting in its pro-inflammatory and anti-inflammatory action can be rather explained by oxidant stress environment and formation of secondary products of NO then by a double nature of NO itself.

Key words: nitric oxide, inflammation, reactive oxygen species, reactive nitrogen oxide species, septic shock

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Introduction

Inflammatory response is one of the phylogenetically oldest responses of the body, which serves the purpose of defending the organism against intruding micro-organisms. Thus, it is a host-defence response, elicited to maintain the integrity of the host. If successful, transient inflammatory response resolves and results in a safe elimination of the micro-organism. However, similarly to some other biological responses, inflammatory response bears a potential of tissue injury. If this tightly regulated inflammatory reaction escapes the control of the host, it may end up with acute reactions, such as hypersensitivity, septic shock or with chronic inflammation, such as rheumatoid arthritis or atherosclerosis. The excessive inflammatory response leads to host tissue injury and may be fatal.

Mediators, which govern the recruitment of inflammatory cells and the intensity of the inflammatory response, include free radicals (such as NO[•], O₂^{•-}), lipid mediators (such as derivatives of arachidonic acid - prostaglandins and leukotrienes), cytokines (such as IL-1, IFN-γ, TNF-α) or protein cascades (such as kinin and complement systems).

Nitric oxide (NO) may represent a good example of a mediator of inflammation, which – as the entire

inflammatory response - is either protective or detrimental to the host. Here, we briefly review biology and biochemistry of NO, and attempt to explain how a single mediator of inflammation, such as NO, may protect the host, kill micro-organisms on one hand, and contribute to the tissue injury of the host on the other.

Biology of NO

Nitric oxide (NO) is a free radical derived from L-arginine through the action of isoforms of nitric oxide synthase. Two of these isoforms are expressed constitutively, i.e. endothelial - eNOS (NOS-3) and neuronal - nNOS (NOS-1) and their activity is crucially dependent on intracellular calcium ions. The third isoform, an inducible one - iNOS (NOS-2) - is a calcium-independent enzyme triggered by proinflammatory stimuli such as endotoxin or cytokines (IL-1, IFN-γ, TNF-α). In biological systems, NO is a short-living molecule with a half-life of about 6 seconds, as it instantly interacts with other free radicals, biological targets or is transformed to nitrite and nitrate [1].

When the production of NO is triggered by physiological stimuli, it evokes its biological effects in cardiovascular and nervous systems mainly by activation of guanylate cyclase, with subsequent elevation of intracellular cGMP [2]. This

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second messenger, activated by endogenous or exogenous NO, leads, for example, to relaxation of vascular smooth muscles, inhibition of leukocyte or platelet adhesion/activation, inhibition of chemotaxis of monocytes, as well as to inhibition of proliferation of vascular smooth muscle cells. Noteworthy, some NO-mediated responses were claimed to be cGMP-independent [3].

NO produced by inducible NOS in higher concentrations than those produced by constitutive NOS isoforms, plays a role in host defence against various pathogens [4]. Toxic antimicrobial and antitumor [5] effects of NO derived from NOS-2 were initially attributed to the direct effect of NO on DNA or other vital macromolecules. At present, it is rather accepted that toxic effects of NO are related to the products of reactions of NO with other free radicals.

Biochemistry of NO

NO is a highly reactive molecule which interacts with oxygen, transition metals, and reactive oxygen species (ROS), especially with superoxide anion [6]. The interaction of NO and O_2^- results in a formation of peroxynitrite $ONOO^-$, that can further decompose into highly toxic products, such as, e.g., hydroxyl radical (OH) [7] or in the presence of thiols it may be transformed to nitrate or S-nitrosothiols [8]. Toxic effects of $ONOO^-$ are related to nitration of tyrosine residues of various functional or structural proteins leading to their irreversible alterations or to irreversible inhibition of mitochondrial respiratory chain [9]. On the other hand, it is currently discussed whether the nitration of proteins is involved in physiological cell signalling [10].

In the cardiovascular system there are numerous enzymatic sources of O_2^- , which could be involved in the formation of $ONOO^-$. These include NADPH oxidase of activated neutrophils, platelets, as well as various enzymes in vessel wall, such as vascular NADPH oxidase, xanthine oxidase or others [11]. Biological activity of O_2^- produced by these enzymes is under control of numerous antioxidant systems such as intra- and extra-cellular SOD as well as catalase, glutathione peroxidase or others [11].

Noteworthy, evidence accumulated suggesting that redox forms of NO, i.e. nitrosonium ion (NO^+) or nitroxyl anion (NO^-), generally described as reactive nitrogen oxide species (RNOS), are much more reactive than NO itself [12]. RNOS avidly react with intracellular and extracellular thiols (glutathione, cysteine residues, albumine). These reactions seem to play an important role in cell signalling as well as in transport of NO in the circulation. Indeed, nitrosonium ion-dependent regulation of gene expression was recently claimed [13], and S-nitroso-albumin was suggested to constitute the reservoir of NO in the circulation [14, 15]. In summary, it is becoming apparent that both biological and toxic effects of NO involve much wider scope of reactions and targets than those related to NO-dependent activation of guanylate cyclase.

NO in chronic inflammation

It is well known that expression of NOS-2 is increased in various inflammatory diseases, such as atherosclerosis, chronic heart disease, host vs. graft disease, rheumatoid arthritis, as well as in numerous neurodegenerative diseases [16]. In all these diseases increased formation of NO was reported as assessed by levels of nitrites and nitrates in plasma, by molecular biology methods as well as by other assays, not always relevant. For example, free 3-nitrotyrosine was found in the serum and synovial fluid of patients suffering of rheumatoid arthritis, and this finding was considered as a marker of increased formation of NO [17]. However, formation of 3-nitrotyrosine results from biological action of secondary reactive nitrogen oxide species ($ONOO^-$) rather than from NO itself. Moreover, human neutrophils are able to nitrate tyrosine, using nitrite as a substrate and myeloperoxidase - the same enzyme that takes part in the process of chlorination of proteins [18]. Accordingly, a level of 3-nitrotyrosine does not reflect NO production, but it may rather mirror the intensity of inflammation.

The major impact of NO on the host in chronic inflammation is related to the fact that it can contribute to the organ injury. In particular, NO is claimed to contribute to damage of the joint cartilage in rheumatoid arthritis, to mucosal injury in inflammatory bowel disease and to degeneration of neurons in neurodegenerative diseases, such as Parkinson's, Huntington's and Alzheimer's diseases [16].

A deleterious action of NO in neurodegenerative diseases is further supported by the use of knockout mice lacking NOS-2. These mice were resistant to the experimentally induced toxicity in central nervous system [16]. Also, NOS-2 inhibitors used in some experimental animal models of chronic inflammatory diseases were able to alleviate the symptoms of inflammation and diminish injury of the inflamed tissue [19].

NO in septic shock

Septic shock and its complications such as ARDS or multiple organ injury represent the extreme clinical outcome of the inflammatory response. Extensive work have been undertaken to clarify the role of NO in this serious disease. Below we present the short overview on the involvement of NO in systemic inflammatory response induced by bacterial endotoxin with particular emphasis on the detrimental and beneficial side of NO action in systemic inflammation.

It is known since a decade that hypotension and vasoplegia (refractoriness to vasoconstrictor agents) in endotoxic shock are mediated by NO produced by NOS-2 induced in vascular wall by LPS or inflammatory cytokines. Indeed, inhibition of NOS-2 in endotoxaemia or septic shock reversed hypotension, and restored vascular responsiveness to vasoconstrictor agents [20]. More recently it was shown that NOS-2 derived NO contributes also to

organ injury in endotoxic shock. In fact, numerous reports demonstrated organ-protective effects of NOS inhibition as they limited lung, liver, kidney or intestine injury [21-24] in endotoxic shock. Similarly to other inflammatory conditions described above, toxic effect of NO was initially attributed to the direct detrimental action of high nanomolar concentration of NO on mitochondrial respiration and DNA structure. Then it was suggested to be rather of indirect nature related to the formation of ONOO⁻. Indeed, endotoxin-induced organ injury was also reduced by SOD mimetics and peroxynitrite decomposition catalyst [24].

Importantly, the belief of the important contribution of NOS-2 derived NO to the pathophysiology of septic shock led to large clinical trials with NOS inhibitors. Their results, however, were disappointing and were prematurely withheld due to the increased trend in mortality of patients treated with L-NMMA, a non-selective NOS inhibitor [25].

Not only in clinical trials but also in the majority of the animal studies non-selective inhibitors, such as L-NAME or L-NMMA, were used in order to inhibit NOS-2, [20, 26]. These agents along with the inhibition of NOS-2 limited at the same time the activity of NOS-3 in endothelium. This is why, after L-NAME-treatment of animals with endotoxic shock, excessive vasoconstriction appeared resulting in subnormal organ perfusion [27], as well as in depression of cardiac output [28], aggravation of liver [29], intestine [30] and lung [31, 32] injury. Clearly, NOS inhibition was not always beneficial, and could be detrimental, leading to the increased mortality rate in endotoxic shock or septic shock [25].

The mechanisms of the protective action of NO generated by NOS-3 in host response to LPS could result from its vasodilator [27], antiplatelet [33] or antileukocyte [34] activities. In studies carried out in the Department of Pharmacology in Krakow, it was shown that NO produced by pulmonary endothelium was a life-saving mediator of endotoxaemic animals by its capability to safeguard pulmonary microcirculation. We have proposed that it is related to the fact that the removal of bacterial LPS from circulation takes place in pulmonary microcirculation and it involves activation and intercellular interactions of sequestered neutrophils and platelets. If this multicellular event mediated by complement-dependent activation of platelets and neutrophils is not tempered by endogenous NO, it is associated with the acute microvascular lung injury and fatal pulmonary oedema [35].

Beneficial effects of NO in endotoxaemia described above depended clearly on NOS-3. However, the growing evidence indicates that NO produced by NOS-2 may also be cytoprotective [36] not only in endotoxic shock but also in other inflammatory diseases as well. In fact, it was recently shown, that selective inhibition of NOS-2 exacerbated the damage of inflamed joint [37].

What mechanisms could be involved in the anti-inflammatory action of NO derived from NOS-3 or NOS-

2? Inhibitory action of NO on platelets and leukocytes is clearly anti-inflammatory, as these cells ignite the inflammation. NO can act as an anti-inflammatory molecule also by its ability to inhibit NF- κ B activation, the major pro-inflammatory transcription factor [38]. Indeed, NF- κ B is involved in inflammatory response of endothelium as it activates the expression of various adhesion molecules (e.g. ICAM, selectin E) and pro-inflammatory cytokines such as IL-6 or IL-8 [39]. Accordingly, one may expect that the major biological role of NO produced by vessel wall, irrespectively of whether by NOS-3 or NOS-2, is to prevent the endothelium from assuming the inflammatory phenotype [40]. Still, the general view is that NOS-2 derived NO is a pro-inflammatory, not anti-inflammatory mediator.

NO regulation in immune system

The literature on the contribution of NO to organ injury in acute or chronic inflammation often neglects the role of NO in direct regulation of mechanisms of innate and acquired immunity. Obviously, induction of NOS-2 in inflammation is regulated by pro-inflammatory cytokines of innate response. On the other hand, there is evidence indicating that endogenous NO released at a site of inflammation by phagocytic cells or from vessel wall may contribute to cross-talk between inflammatory cells (innate immunity) and Th1/Th2 cells (acquired immunity) and even to fine tuning of Th1/Th2 immune response.

Indeed, it is commonly accepted that Th1 type cytokines, especially IFN- γ , induce the expression of NOS-2 and enhance the synthesis of NO. Conversely, the production of NO can be inhibited by Th2 type cytokines, such as IL-4 and IL-10. This may occur at two levels. First, it can be blocked directly, as these cytokines inhibit NOS-2 transcription in target cells. Second, it can be inhibited indirectly, since IL-4 and IL-10 diminish production of IFN- γ by inflammatory cells.

On the other hand, exogenous NO regulates *in vitro* cytokine production by T cells, though the profile of its activity is still not clear. It is generally accepted that NO at a high concentration is immune-suppressive. Indeed, some authors [41] show indiscriminate inhibition of production of Th1 and Th2 type of cytokines (IL-2, IL-4, IL-5, IL-10 and IFN- γ) by NO-donors. Others suggest that NO donors inhibit production of Th1 type cytokines (IFN- γ , IL-2), but not those of Th2 type (IL-4, IL-10) [42].

To complicate the matters further, recently it was demonstrated that low concentrations of NO selectively enhance the differentiation of Th1, but not Th2 cells. The authors show that NO, in a cGMP-dependent way, induced the expression of IL-12 receptor, a key cytokine in induction of Th1 differentiation [43].

In spite of these controversies, which may result from the different experimental systems, the contribution of NO to regulation of cytokine generation seems to be documented. Yet, many questions are left unsolved.

In summary, NO displays multiple actions in inflammatory response. Importantly, it protects the host and contributes to the tissue injury of the host. The classical view that protective vs. detrimental nature of NO depends on the source of its production (NOS-3 vs. NOS-2) seems to be no longer tenable. The detrimental role of NO may be rather explained by the formation of secondary products of NO in oxidant stress environment. Accordingly, the pharmacotherapy of inflammatory diseases should perhaps aim at the restoration of NO protective properties and at the inhibition of deleterious effects of reactive species of oxygen and nitrogen. Whether this approach will bring beneficial modulation of Th1/Th2 balance remains to be determined.

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Abbreviations

IL – interleukin

IFN – interferon

TNF – tumor necrosis factor

SOD – superoxide dismutase

ARDS – adult respiratory distress syndrome

LPS – lipopolysaccharide

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