Section A -Research paper

Role of Nitric Oxide as an Inflammatory Marker

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Abstract:

Nitric oxide (NO) is a signaling molecule that plays a key role in the pathogenesis of inflammation. It gives an anti-inflammatory effect under normal physiological conditions. On the other hand, NO is considered as a pro-inflammatory mediator that induces inflammation due to over production in abnormal situations.

Keywords: NO, inflammation, MPTP.

Introduction

Nitric oxide (NO) is best known for its actions in the vasculature. In addition, NO plays a key role in cell metabolism and is instrumental in coordinating tissue energy demand with supply. Physiologic NO signaling is pivotal to metabolic and cardiovascular homeostasis. Dysregulation of NO signaling pathways is associated with the pathogenesis of cardiometabolic disorders **(1).**

Nitric Oxide Synthase

NO is produced in many tissues by four distinct isoforms of NO synthase (NOS):

(1) neuronal NOS-1 (nNOS),

(2) inducible NOS-2 (iNOS),

(3) endothelial NOS-3 (eNOS) and

(4) mitochondrial NOS (mtNOS) **(2).**

Whereas iNOS is inducible, eNOS and nNOS are constitutively expressed, continuously elaborating NO. NOS tissue expression is less strict than implied by the nomenclature, and all three isoforms may be constitutive or inducible **(2).**

NOS consists of a reductase and oxygenase domain. Coupling of the reductase domain of one NOS monomer with the oxygenase domain of its partner is required for proper NO production. The NOS dimer requires nicotinamide adenine dinucleotide phosphate oxidase (NADH/NADPH), tetrahydrobiopterin (BH4) cofactor and oxygen (O2) to convert its substrate, L-arginine, to L-citrulline, with the release of the oxidized nitrogen terminal of L-arginine, NO **(2):** (NOS dimer)

L-arginine → L-citrulline + NO·

Molecular O2, rather than L-arginine, becomes the substrate for the uncoupled NOS monomer, generating superoxide O2–· in lieu of NO, thus increasing prooxidant stress.

Nitric Oxide Signaling

NO Bioavailability

NO is a structurally simple, low-molecular-weight, highly lipophilic free radical. It is extremely reactive, readily forming other nitrogen oxides, which curtails NO bioavailability temporally and spatially:

- NO has a very short half-life;
- NO can travel only limited distances before being oxidized **(3).**

Nitrite and nitrate NO reaction products, derivative S- or N-nitrosoproteins and iron-nitrosyl complexes, are not just inert metabolic waste products. They can be reduced back to release free NO via several pathways **(3).**

NO bioavailability thus resides not only in the NO radical, but also in NO-containing compounds. These NO products serve as storage pools of bioactive NO and appear to participate in NO-related processes as they, in contrast to NO, can travel via the circulation to remote tissues**(3).**

Intracellular Signalosome

Cytosolic oxidants limit NO bioactivity even intracellularly, foiling its diffusion to molecular targets more than approximately 100 µm removed from NOS. This restricted diffusion, combined with the specific subcellular localizations of NOS, confers specificity and efficiency to NO signaling by confining its actions to protein targets colocalized with NOS within complex multiprotein signalosomes **(4).**

NO Signaling

NO signals via three mechanisms:

(1)Guanylate cyclase activation. By binding to its heme group, NO activates soluble guanylate cyclase, which produces 3′-5′-cyclic guanosine monophosphate (cGMP) from guanosine 5′-triphosphate (GTP), the amount generated being proportional to the amount of NO. cGMP activates protein kinase G (cGK) as downstream effector **(5):** (NO:guanylate cyclase)

GTP → cGMP → activated cGK effector

(2)S-Nitrosylation. NO covalently and reversibly forms S-nitrosothiol groups with reactive cysteine thiols in a wide range of target proteins **(6).**

(3)Mitogen-activated protein kinases (MAPKs). The intracellular formation of peroxynitrite leads to activation of MAPKs.

Most NO effects are mediated via S-nitrosylation in a cGMP-independent manner **(6).**

Nitric Oxide Functions

Mitochondria

NO effects on mitochondria have considerable implications for cell physiology and cell death. Mitochondria are primary cellular targets for NO.

mtNOS is linked to mitochondria at several sites of the mitochondrial electron transport chain (ETC), most notably at Complex I (NADH dehydrogenase) and Complex IV (cytochrome c oxidase, CcOX) **(7).**

mtNOS is highly activated by activation of the ETC and Complex I, which serves as its source of electrons to produce NO. Conversely, inactivation of Complex I terminates normal mtNOS activity **(8).**

Metabolism

mtNOS-derived NO effectively controls mitochondrial respiration, O2 consumption, transmembrane proton gradient and potential and adenosine triphosphate (ATP) synthesis**(7).**

Acutely, NO reduces mitochondrial oxidative metabolism **(9).**

Cell Protection

Ischemic preconditioning provides powerful cardioprotection against myocardial ischemia-reperfusion injury. Physiologic NO levels are involved in cytoprotective effects of early and late preconditioning. Not only eNOS-, but also exogenous nitrate-donor-derived NO can effect endothelial and myocardial cytoprotection **(10).**

NO/cGMP may protect against mitochondrial permeability transition and apoptosis induced by manifold insults. Through its interaction with ETC components, such as CcOX, NO affects low-level ROS generation and other mitochondrial defense mechanisms, thereby triggering adaptive cell survival signaling**(9).**

Anti-Inflammatory and Antiatherogenic Activities

Physiologic NO levels are anti-inflammatory. By preventing proinflammatory cytokine activation, NO protects blood vessels from endogenous injury, interfering with early and later stages of conduit vessel atherogenesis **(11).**

- NO delays endothelial cell senescence and senescence-related proinflammatory signaling,
- NO reduces endothelial cell apoptosis,
- NO inhibits the transcription of nuclear factor-κB,
- NO inhibits redox-sensitive, cytokine-induced vascular cell adhesion molecule-1, intracellular adhesion molecule-1 and monocyte chemoattractant protein-1, preventing leukocyte adhesion to the endothelium,
- NO decreases endothelial permeability, reducing the influx of oxidized lipoproteins into the vascular wall,
- NO interferes with leukocyte migration into the vascular wall by decreasing the expression of factors, including the surface adhesion molecules CD11/CD18 and P-selectin,
- NO powerfully inhibits inflammatory cell activation and monocyte activity.
- NO inhibits the synthesis and secretion of extracellular matrix proteinases, which degrade extracellular matrix proteins,
- NO increases the expression of tissue inhibitor of matrix metalloproteinases,
- NO inhibits transforming growth factor-β/Smad-regulated gene transactivation **(12).**

Table 3: Factors that reduce NO bioavailability

Hypertension Dyslipidemia Free fatty acids Hyperuricemia Angiotensin II NADH/NADPH activation Proinflammatory cytokines Glucose intolerance	decreased NO production/activity

Manifestations of Reduced NO

Reduced physiologic NO signaling and increased superoxide formation by

dysfunctional NOS are pathogenic and contribute to the clinical course of cardiometabolic disease **(13).**

Inflammation

Impaired NO bioavailability promotes inflammation. Vascular inflammation is increased in eNOS(–/–) mice. Upregulation and activation of nuclear factor-κB and activator prote-in-1 initiate the release of inflammatory cytokines, such as TNF-α and interleukin-1. As T lymphocytes migrate into the vascular intima, they produce further cyto- and chemotactic factors, as well as adhesion molecules, to recruit VSMCs and monocytes, initiating atherogenesis**(14).**

Role of NO in Physiology and Pathophysiology

NO is a key molecule involved in a variety of biological functions throughout the whole body. In the vasculature, NO (major part from NOS3, but NOS1 is present around arterioles) regulates vascular tone and blood flow by activating soluble guanylate cyclase (sGC) in the vascular smooth muscle. Moreover, it is essential for leucocyte adhesion and platelet aggregation, and it controls mitochondrial oxygen consumption by inhibiting cytochrome c oxydase. Abnormalities in vascular NO production and transport result in endothelial dysfunction with various cardiovascular pathologies like hypertension, atherosclerosis and angiogenesis-associated disorders. Interestingly, NOS3 can generate superoxide when the concentrations of either L-arginine or BH4 are low. This “uncoupling” of NOS3 occurs in several pathologies, like diabetes, hypercholesterolaemia and hypertension. NO production was also suggested as a major inherited factor of insulin sensitivity, with diet-induced oxidative scavenging of NO as a first hit towards insulin resistance **(15).**

NO in the brain regulates many physiological processes affecting behavior and cognitive function, including synaptic plasticity. In addition, it also controls brain blood flow, promotes angiogenesis, maintains cellular redox state, cell immunity and neuronal survival. Its over-production may lead to neurodegeneration **(16).**

NO has a complex and multifaceted role in inflammation

NO is produced by two constitutive forms of NOS, neuronal (NOS-1/nNOS) and endothelial (NOS-3/eNOS), and one inducible form (NOS-2/iNOS). Typically, the constitutive forms of NOS produce tonic, pulsatile volumes of NO, while iNOS produces the high volumes that are synonymous with inflammation. Namely, iNOS produces more (micromolar) NO for longer (hours) compared to eNOS and nNOS that produce less NO (nanomolar amounts) for shorter periods (seconds to minutes) (**17).**

In inflammation, NO acts as a vasodilator and increases leukocyte adherence to the endothelium of blood vessels. NO also increases the permeability of blood vessel walls, allowing leukocyte transmigration into extravascular spaces. The levels of NO and the mode of its secretion (tonic/low and continuous or acute/high and transient) influence the downstream functions of NO. For instance, in physiological conditions, the low and constant levels of NO produced by eNOS allow white blood cells to remain suspended in blood as this basal level of NO inhibits

leukocyte adhesion. However, during inflammation, eNOS contributes to an acute increase in NO, removing the inhibition on leukocyte adhesion and increasing diapedesis. Therefore, NO has a dual role in leukocyte adhesion that is dependent on the levels that are produced (**18).**

Deletion of the eNOS gene results in a dramatic drop in NO, implicating eNOS as the main contributor to VEGF-induced NO production. It is important to note that in human endothelial cells, VEGF induced only eNOS and not iNOS, indicating different functional importance of NOS isoforms between species. The contribution of each NOS isozyme in inflammatory conditions is therefore highly context-dependent, and selective modulation of NOS isoforms is important to consider in therapeutics. If NOS inhibitors are to be used in therapy, the pathophysiology of NO-mediated effects must be accurately determined, including the forms of NOS involved and concentrations of NO produced, to prevent tipping the balance towards deleterious inflammatory consequences (**17).**

NO is implicated in the pathophysiology of neuroinflammatory diseases

Excessive NO production in neuroinflammation is now recognized as an important pathological component of diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD) and modulating NO in these diseases would be beneficial. Accumulation of beta-amyloid (Aβ) plaques and neurofibrillary tangles are characteristic of AD pathology. Kummer et al. showed that products of iNOS activation such as NO and $ONOO^-$ cause nitrotyrosination of Aβ42, accelerating their aggregation into amyloid plaques. (**19**)

For PD, the prodrug 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) models the dopaminergic damage in the substantia nigra and striatum. Inhibiting iNOS with S-methylisothiourea (SMT) increased substantia nigral dopaminergic neuron number, decreased nitrate/nitrite levels, decreased lipid peroxidation, and reduced caspase-3 activity in MPTP-treated mice. Importantly, inhibiting iNOS reduced signs of bradykinesia. Another common PD model is the 6-hydroxydopamine (6OHDA) mouse model. In this model, motor impairment can be assessed by the amphetamine rotation test. Studies have shown that administration of NG-nitro-L-arginine methyl ester (L-NAME), a non-specific NOS inhibitor, in 6OHDA mice inhibited amphetamine-induced rotation, alongside improved levels of dopamine and its metabolites. The iNOS inhibitor GW274150 also improved outcome in 6OHDA mice, with reduced tyrosine hydroxylase (TH)-positive neuron loss in the substantia nigra (**20).**

Nitric oxide in neurodegeneration and mechanism

There are several important roles of NO in the peripheral and central nervous systems. It has a role in both neuroprotection and neurotoxicity, the presence of eNOS is found in the vascular endothelium, which is involved in the regulation of blood flow, decrease neuronal apoptosis, and platelet aggregation. It was also reported that neuronal relaxation is mimicked by agents producing NO, supported by the data that nerve mediated gut relaxation is prevented by NOS inhibitors. Numerous studies, few

on autopsied human brains, have demonstrated a significant involvement of NO in neurodegenerative disorders. Decreased levels of endothelial NO play a key role in the upregulation of Aβ expression and modulation of amyloid precursor protein (APP) in the cerebrovasculature. Utilizing anti-3-nitrotyrosine polyclonal antibody-mediated immunolabelling, Duda and coworkers demonstrated a widespread nitration of Lewy bodies and Lewy nitrites in the autopsied cortex of patients with Lewy bodies and Alzheimer's disease. Furthermore, nitration of α-synucleins in glial cells of the autopsied cerebellar white matter, in patients of multiple system atrophy, and nitration of Lewy body-like inclusions and neuroaxonal spheroids in the autopsied globus pallidus was found in patients with neurodegeneration with brain iron accumulation type 1 **(21).**

The involvement of NO and its reaction product (with superoxide radicals), peroxynitrite, in AD pathology has also been reported from post-mortem studies on AD afflicted human brains as exceptionally raised levels of nitration of neurofibrillary tangles were evident in the hippocampus of AD patients as compared to the age-matched controls. Some studies, based on animal models of 1-methyl 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (neurotoxin responsible for inhibition of mitochondrial respiratory chain complex I and imitates PD symptoms through degeneration of substantia nigra neurons)-induced neurotoxicity, showed that NOS inhibition delayed the progression of disease pathology. The involvement of NO and peroxynitrite was also reported from post-mortem studies of PD afflicted brains wherein elevated nitration of tyrosine residues in the degenerating neurons of substantia nigra pars compacta was reported. Additionally, nitration of tyrosine residues of proteins has emerged as a crucial factor in the pathogenesis of a wide array of neurodegenerative disorders. It is also interesting to note here that a majority of neurodegenerative pathogenicities, mediated by NO, are through nitration (**22**).

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