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Potential Immunoregulatory and Antiviral/SARS-CoV-2 Activities of Nitric Oxide

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Nitric oxide (NO) represents a key signaling molecule in multiple regulatory pathways underlying vascular, metabolic, immune, and neurological function across animal phyla.

Our brief critical discussion is focused on the multiple roles of the NO signaling pathways in the maintenance of basal physiological states of readiness in diverse cell types mediating innate immunological functions and in the facilitation of proinflammatory-mediated adaptive immunological responses associated with viral infections. Prior studies have reinforced the critical importance of constitutive NO signaling pathways in the homeostatic maintenance of the vascular endothelium, and state-dependent changes in innate immunological responses have been associated with a functional override of NO-mediated inhibitory tone. Accordingly, convergent lines of evidence suggest that dysregulation of NO signaling pathways, as well as canonical oxidative effects of inducible NO, may provide a permissive cellular environment for viral entry and replication. In immunologically compromised individuals, functional override and chronic rundown of inhibitory NO signaling systems promote aberrant expression of unregulated proinflammatory pathways resulting in widespread metabolic insufficiencies and structural damage to autonomous cellular and organ structures. We contend that restoration of normative NO tone via combined pharmaceutical, dietary, or complex behavioral interventions may partially reverse deleterious physiological conditions brought about by viral infection linked to unregulated adaptive immune responses.

MeSH Keywords: **COVID-19 • Inflammation • Mitochondria • Nitric Acid**

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Numerous studies in the biomedical literature have attempted to elucidate the multifaceted functional roles of the free radical gas nitric oxide (NO) as a key signaling molecule in multiple regulatory pathways underlying vascular, metabolic, immune, and neurological function across animal phyla [1,2]. The widely studied biosynthetic pathway for NO production utilizes the guanidino group of L-arginine as its obligate chemical donor within a series of intermediary enzymatic steps catalyzed by 3 tissue-specific isoforms of NO synthase (NOS): endothelial (eNOS), neuronal (nNOS), and inducible (iNOS) [1]. The eNOS and nNOS isoforms are constitutively expressed and strictly regulated to deliver short-lived bursts of released NO, whereas iNOS is induced following a proinflammatory/microbial challenge and is designed to deliver continuous high concentrations of NO to chemically neutralize invading pathogens via a canonical oxidative pathway [1]. Historically, the chemically-mediated antibacterial and antiviral actions of NO have been attributed to the local actions of iNOS, and research has not adequately focused on more adaptive, state-dependent changes in constitutively expressed NOS within integrated immune-regulatory signaling systems [3]. We contend that despite a clear divergence in catalytic endpoints mediated by constitutive NO and iNOS, it appears that the overall goal of concerted NO production and release by multiple isoforms is to maintain homeostasis of integrated cellular and multiorgan systems in response to chemical or biological agents [2].

We have previously addressed a compelling biomedical hypothesis stating that constitutive NOS activity is a key regulatory factor responsible for maintaining basal physiological states of tonic inhibition and dampened micro-environmental noise within diverse cell types and integrated organ systems [2]. Conversely, physiologically evoked cellular activation is proposed to occur via a coordinated series of disinhibition processes that are in part functionally linked to alterations in patterning of constitutive NO production and release. From a timely immunological perspective, biphasic effects of constitutive NO production and release have been observed to differentially affect biochemical activation and intra-nuclear transport of the master transcriptional regulatory complex NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) [2,4]. The critical role of NF- κ B as a rapid transcriptional activator in response to a wide spectrum of biological and chemical threats is reflected by its diversity of molecular targets in the mediation of innate and adaptive immunological responses, notably functional mosaics of proinflammatory cytokines associated with viral infections. A recent study has demonstrated a potentially important regulatory role of inducible NO/iNOS expression on the epigenetically mediated nuclear reprogramming of stem cells to pluripotent stem cells that are mediated in part by S-nitrosylation of nuclear proteins [5]. Interestingly, the authors have observed that the graded response of innate immune activation is determined by the degree of activation of NF- κ B and is defined by the maximal yield of induced pluripotent stem cells. These observations

are consistent with those of an earlier study demonstrating canonical oxidative effects of inducible NO that were functionally linked to enhanced transcriptional activation of cytokine genes following group B streptococcus infection [6]. Finally, we hypothesize that complex, temporally-defined patterns of released NO from constitutive NOS isoforms in concert with significantly higher concentrations of NO produced by iNOS may contribute to downstream anti-microbial/anti-viral effects of interferons (IFNs) and IFN-inducible effector proteins in processes defined as cell-autonomous immunity [7].

Preclinical and clinical studies have reinforced the critical importance of constitutive NO/eNOS signaling pathways for homeostatic maintenance of the vascular endothelium [8]. Normative control of pulsatile blood flow within the vasculature and extending into the microvasculature are mediated by concerted release of constitutive NO and functionally interactive autacoids that include prostacyclin and endothelium-derived hyperpolarizing factor [9]. The observed reciprocal regulatory functions of constitutive NO and endothelium-derived hyperpolarizing factor within the vascular endothelium are mediated at the luminal cell surface via mechano-transductive events linked to minute changes in hemodynamic flow. Collected data lend empirical support for a short-regulatory circuit whereby physiologically relevant hemodynamic events promote activation of eNOS via dissociation from caveolin within caveolar scaffolds followed by functional coupling to calmodulin [8–10]. Within this proposed model, the calcium dependency of NO formation by eNOS indicates that normative oscillations/pulsations in cytosolic calcium flux, notably within the endoplasmic reticulum-mitochondrial axis, may be functionally coupled to a patterned rhythmicity of NO release responsible for maintaining basal physiological states of tonic inhibition [11]. State dependent changes in innate immunological responses have been associated with a functional override of NO-mediated inhibitory tone within the vascular endothelium as reflected by immunocyte adherence, endothelial shape changes, and loss of integrity of gap junctions in juxtaposed cells [12]. Finally, under surgical conditions involved with saphenous vein conduits in coronary revascularization, increased pressure and cyclic distention lead to impaired NO production and increased immunocyte adhesion, thereby mimicking a proinflammatory physiological state [13,14].

As discussed, convergent lines of evidence suggest that dysregulation of NO signaling pathways as well as canonical oxidative effects of inducible NO may provide a permissive cellular environment for viral entry and replication. We have previously observed that *in vitro* exposure of primary cultures of vascular endothelial explants to human immunodeficiency virus envelope protein gp120 promoted proinflammatory monocyte adhesion [15–17]. Importantly, the inhibitory effects of a pharmacologically administered NO donor on gp120-mediated monocyte adhesion were observed to undergo significant desensitization.

This effect is consistent with our contention that disruption of tonic NO inhibitory tone following xenobiotic chemical or pathogenic stimuli represents an activation mechanism functionally linked to the initiation of proinflammatory cellular defense mechanisms with subsequent transcriptional activation of multiple cytokine signaling pathways [6,7]. Interestingly, non-canonical oxidative effects of NO were observed to inhibit the replication of SARS-CoV-2 by 2 proposed mechanisms: 1) reduced palmitoylation of nascently expressed spike (S) protein which effectively diminishes the affinity of the viral receptor binding domain to ACE2, and 2) chemical modification/inactivation of one or both of the cysteine proteases encoded by Orf1a of SARS-CoV-2 [18]. In this regard, an immune vaccine strategy has been reviewed by our group [19]. We therefore contend that in immunologically compromised individuals, functional override and chronic rundown of inhibitory NO signaling systems promote aberrant expression of unregulated proinflammatory pathways resulting in widespread metabolic insufficiencies and structural damage to autonomous cellular and organ structures. Thus, permissive facilitation of viral entry and replication is predictably linked to exponential expansion of proinflammatory responses in the absence of potent modulatory effects of NO signaling pathways [20]. Restoration of normative NO tone via combined pharmaceutical, dietary, or complex behavioral interventions may partially reverse deleterious physiological conditions brought about by viral infection linked to unregulated adaptive immune responses [21].

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Furthermore, these restorative processes may be incorporated into reinforced mind-body activities, notably the relaxation response, which promote whole body metabolic advantage via systemic downregulation of cellular processes and enhanced mitochondrial bioenergetics [22]. We have previously proposed and discussed a unified mechanism of action linking behaviorally mediated enhancements of whole-body metabolic advantage to optimization of synchronous regulation of mitochondrial oxygen utilization by intra-mitochondrial NO production and release [22].

Conclusions

In summary, preemptive enhancement of whole-body NO tone may represent a novel approach to optimize human health by incorporating both behavioral, physical, and pharmaceutical approaches. Similar preemptive approaches may be developed that specifically maintain tonic inhibitory NO tone as a novel approach to lessen deleterious physiological conditions associated with viral infection and rampant proinflammatory processes.

Conflict of interest

None.