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Nitric Oxide: Role in Human Biology

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ABSTRACT
Nitric oxide (NO), a free radical, possesses various modulatory effects on biological systems. NO is synthesized from L-arginine by converting it to L-citrulline via nitric oxide synthase (NOS) enzymes. Moreover, various precursors of NO have been reported that include arginine, citruline, arginine alphaketoglutarate (A-AKG) and argineneketoisocaproate (A-KIC). NO possess various direct and indirect effects that broadly affect various tissues and organ systems inside the body. The present review article aims to discuss about the pharmacology, physiological roles and effects of NO.

Keywords: Nitric oxide, modulatory effects, nitric oxide synthase, precursors.

INTRODUCTION

Nitric oxide (NO), a colorless gas, has been considered as an important biological regulator which is a fundamental component in the fields of neuroscience, physiology and immunology. [1-2] The term NO was first termed in 1772 by Joseph Pristley when he named it as 'nitrous air'. It was known as a toxic gas and an air pollutant till 1987, when it was shown that it is actually produced naturally in the body and thus its role has been described in the regulation of blood pressure and protection from various cardiovascular diseases. [3] Moreover, the cardioprotective roles of NO regulation include blood pressure and vascular tone, inhibition of platelet aggregation and leukocyte adhesion along with prevention of smooth muscle proliferation. [4-6] In addition, it has been comprehensively demonstrated that reduced bioavailability of NO leads to development and progression of various cardiovascular disease. [7] Further, the reactivity of NO depends on various physical parameters like small size, high diffusion rate and lipophilicity. [8] NO has been shown to possess various direct and indirect effects leading to its effect on various biological systems. However, it has been noted that NO can protect cells at low level but it is implicated in tumor angiogenesis and progression at higher levels. [9-10]

The present review article explains about the synthesis and physiological roles possessed by NO in living systems. Moreover, the effects of NO have been critically discussed in the present review article.

NITRIC OXIDE SYNTHESIS

NO is produced by various group of enzyme termed as nitric oxide syntheses (NOS) which are present in body. [11-12] The synthesis of NO takes place by the conversion of L-arginine to L-citrulline, the reaction being catalyzed by nitric oxide synthases (NOS). [13] The two cofactors have been found to be involved in this process which includes oxygen and NADPH. Moreover, three isoforms of NOS are present whose names are termed on the basis of their activities, which include following neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS). [13-14] Furthermore, scientists have represented their names by numbers like NOS 1 for nNOS, NOS 2 for iNOS and NOS 3 for eNOS. The isoforms of NOS can be broadly categorised as constitutive (cNOS) and inducible NOS. the cNOS is calcium-dependent and continuously present whereas iNOS is Ca^{2+} independent and has been found to be expressed only after cytokine exposure. [15] Based on this category nNOS and eNOS are constitutively expressed and require elevated levels of Ca^{2+} alongwith activation of calmodulin in order to produce NO for short period of time. In addition, it has been shown that nNOS and eNOS synthesize NO in response to intracellular Ca^{2+} levels, whereas the reactivity of NOS isoform depend upon their binding with calmodulin. [15-16] When intracellular Ca^{2+} level is leads to increased production of calmodulin ultimetly leading to augmented binding of calmodulin to eNOS and nNOS which further leads to enhanced production of NO by their enzymes. [17]

PHARMACOLOGY OF NO

NO, a binary molecule with chemical formula NO, is a free radical which has been an important intermediate in the chemical industry. [18-19] NO is a byproduct of combustion of substances in automobile engines, fossil fuel power plants. In addition, it is produced naturally during the electrical

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discharges of lightning in thunderstorms. Several mechanisms have been proposed by which NO affects the biology of living cells which include oxidation of iron-containing proteins such as ribonucleotide reductase and aconitase, activation of the soluble guanylate cyclase, ADP ribosylation of proteins, protein sulfhydryl group nitrosylation and iron regulatory factor activation. [20] Moreover, NO has been well demonstrated to activate nuclear factor kappa-B (NF-κB), an important transcription factor, in iNOS gene expression in response to inflammation. [21] Furthermore, NO has been found to act through the stimulation of soluble guanylate cyclase, a heterodimeric enzyme with subsequent formation of cyclic GMP. The cGMP has been further noted to activate protein kinase G (PKG) causing phosphorylation of myosin light chain phosphatase (MLCP). [22] The inactivation of MLC-kinase ultimately leads to the dephosphorylation of the myosin light chain (MLC), causing smooth muscle relaxation. [23]

NO has been potentially regarded as an antianginal drug which causes vasodilation, ultimately helping the ischemic pain of angina by decreasing cardiac workload. Moreover, NO drugs lower arterial pressure and left ventricular filling pressure by the dilatation of veins. [24] This vasodilation decreases the force which the cardiac muscle exerts to pump the equivalent volume of blood. In addition, the nitroglycerin tablets are taken sublingually which have been successfully noted to prevent the acute chest pain by mechanism in which nitroglycerin reacts with a sulfhydryl group (–SH) in order to produce NO that ultimately reduces the pain by causing vasodilation. [25-26] Furthermore, nitrates have been reported to possess beneficial roles for the treatment of angina due to reduced myocardial oxygen consumption both by decreasing preload and afterload of coronary vessels. [24]

Moreover, various precursors of NO have been reported like Arginine, citruline, A-AKG and A-KIC. The main role of L-arginine is to act as an important precursor for production of NO which is evidenced by the fact that supplement source of L-arginine production ultimately leads to enhanced NO production. [27] Citruline, another precursor to arginine, has the ability to increase plasma levels of arginine endogenously in human body ultimately increasing NO production. Further, both AKG and KIC have been known as independent precursors when compared to L-arginine which aids in enhanced NO production. Both experimental and clinical data suggests that AKG is significantly involved in enhanced NO production. Moreover, KIC has been shown to possess nitrogen sparing effects by inhibiting the breakdown of muscle protein. [28] Furthermore, studies describe that AKG decreases lactic acid in the cell mitochondria by increasing the levels of oxygen uptake.

BIOLOGICAL REACTIONS OF NO

The biological reactions of NO have been divided into three main parts including diffusion, Auto oxidation to form nitrous anhydride (N_2O_3) and reaction with superoxide (O^{2-}) to form peroxynitrile ($ONOO^-$). [29-30] NO has been noted to enter the cell membrane by simple diffusion and reacts with cellular component. Once it enters the cell, it reacts with non-heme iron or quench tyrosyl radical of ribonucleotide reductase which may lead to inhibition of DNA synthesis. [31] Moreover, NO reacts with nitrogen dioxide (NO_2) in order to form N_2O_3.

$$NO + NO_2 \longleftrightarrow N_2O_3 \longleftrightarrow 2NO_2^- + 2H^+ \quad \{1\}$$

Further, NO reacts with O^{2-} to form $ONOO^-$, a potent oxidant which reacts with almost all biological molecules. [32] The peroxynitrite anion combine with CO_2 in order to form nitrosoperoxy carbonate adduct, the reaction referred to fasted reaction, which on decomposition leads to the formation NO^{3-} and CO_2.

$$ONOO^- + CO_2 \longrightarrow [ONO_2.CO_2^-] \longrightarrow NO^{3-} + CO_2 \{2\}$$

In addition, NO reacts with molecular oxygen, the reaction takes place in aqueous or in gas phase. However, the rate of the reaction is second order with respect to NO but first order with respect to O_2. Further, NO_2 is a stable product of NO oxidation in gaseous phase, whereas, NO_2 gives rise to NO and NO_3^- in aqueous solutions. Ultimately, NO reacts with molecular oxygen in order to form peroxynitrite. [33-34]

$$2NO + O_2 \longrightarrow 2NO_2 \longrightarrow NO^- + NO_3 \longrightarrow ONOO^-$$

PHYSIOLOGICAL ROLE OF NO

Initially, NO has been reported to exert various physiological roles due to its ability to induce vasodilatation. [35-36] By the time, numbers of other physiological roles of NO have been demonstrated that include its role in immune system, nervous system, inflammation and blood flow. NO has been produced by numerous cells which are involved in the immune response. In particular, cytokine-activated macrophages have been noted to produce high levels of NO which is involved in the killing of targeted cells as tumors and bacteria. [37] Moreover, NO acts as a mediator in inflammatory process by which NO enhanced the cyclo-oxygenase (COX) enzyme's effect ultimately leading in increased production of proinflammatory eiconosids. [38] Further, the role of NO in nervous system has been evidenced by the fact that NO behaves as neurotransmitter in the cerebral and peripheral nervous system by which NO is involved in the regulation of apoptosis in neurons. [38-39] Furthermore, NO has been noted to relax the smooth muscle and walls of arterioles. The complex endothelial cells lining the blood vessels release a puff of NO at each systole, which gets diffused into the underlying smooth muscle cell, and thus permits the surge of blood to pass through easily. [40-41]

EFFECTS OF NO

NO has been shown to exert various biological effects that can be categorized into direct effects, indirect effects and other effects. [42-43] It has been comprehensively reported that NO possesses indirect effects at low concentration but the direct actions will be shown at higher concentration. NO reacts rapidly with metal complexes to form metal nitosyls like Fe-NO complex which is a stable product. [44] Moreover, NO has the capability to react with metelloxo and metal oxo complexes. NO directly reacts with hypervalent complex in presence of some agent like hydrogen peroxide (H_2O_2) in order to reduce to lower valancy states. Further, the presence of NO results in scavenging O^{2-} ions which converts any ferrous oxy adducts to active ferric state. In addition, NO has been noted to prevent peroxide mediated tissue damage by scavenging metal oxo species. [44-45] It inhibits lipid oxygenase activity by reacting with non heme iron at the active site. It has also been seen that NO generation leads to nitro active action at nucleophilic centres resulting in the formation of S-nitrosothiols. Further, the excess production of NO has been noted to cause glutamate induced neuronal toxicity in cortical and striatal neurons culture. NO plays modulatory roles in fighting against cardiovascular diseases

by improving blood supply to the cardiac muscle. [46] The effect of NO in brain has been proved by the fact that after the release from synapses, NO remains only for few seconds and then immediately coupled due to its rapid diffusion. NO has also been shown to inhibit cytochrome c (cyt c) in their micro molecular range which ultimately leads to leakage of O^{2-} from electron transport chain (ETC). [47] The leakage of NO from ETC leads to damage of DNA by three mechanisms that include formation of nitrosamine, inhibition of DNA lesion repair system, and modification of DNA due to its oxidative products.

The indirect effects of NO have been produced due to the reactions between O^{2-} and NO which leads to the production of $ONOO^-$. Further, $ONOO^-$ gets converted to NO_2, an inactive entity, in the presence of excess NO or superoxide. [44] It has also been demonstrated that catechol-estrogens complexes are oxidized to quinones which reduce oxygen to generate O^{2-} ion. Similarly, polyhydroxy aromatic compounds like pyrogallol and 1, 4-hydroquinone autooxidize form semiquinone radicals that react with O_2 to generate O^{2-} which in combination with various NO releasing compounds results in production of $ONOO^-$ which is responsible for DNA damage. $ONOO^-$ also leads to increased protein and enzyme function by the nitration of tyrosine residue in tissue which ultimately leads to pathological dysfunction. [48] The formation of 3-nitrotyrosine is contributed by many nitrogen oxide species like $ONOO^-$, NO_2, nitrous acid and nitronium ion. The 3-nitrotyrosine has been found to play modulatory roles in the pathogenesis of various diseases like chronic inflammation, atherosclerosis and acute lung injury. Moreover, other indirect effects of NO can be divided as oxidation and nitrosation. [49] When removal of electrons or hydroxylation reactions occurs, similar to those for reactive oxygen species (ROS), leading to oxidative stress, the condition is termed as oxidation reactions, whereas, the reactions in which reactive nitrogen oxide species (RNOS) donate NO to nucleophilic groups like thiols and amines, lead to formation of nitrosonium adducts, referred to as nitrosation reactions and the condition is termed as nitrosative stress. [50]

As the direct or indirect effects of NO leads to DNA damage and modulate the biological molecule; however other effects of NO have also been comprehensively reported. The other potent effects of NO include antitumor effects, effects on mitochondria and effect with metal ions. [51] Studies have shown NO to possess capability to eliminate intracellular pathogen and block the viral replication. No has been demonstrated to act on p53 gene by upregulating its expression, which have tumor suppressor property, evidencing its role as antitumor effect. [52] In addition, NO has been noted to inhibit DNA damage induced by ROS and RNOS. Further, NO donors possess various properties like inhibiting angiogenesis, metastatis and tumor growth, which further supports its antitumor effects. [53] Moreover, at high risk carcinogenic sites, a prolonged expression of iNOS during chronic inflammation has been noted which leads to enhanced generation of NO from macrophages, kupffer cells and NK cells ultimately leading to inhibition of replication, which further evidenced its antitumor effect at the target cells. [53-54] It has been expansively reported that NO protect the tissue from peroxide mediated damage which happen due to scavenging oxo species. Additionally, NO has the ability to inhibit metastasis by reducing intracellular store of GSH or by blocking the adhesion of tumor cell on venular side of microcirculation. [55] Further, liver endothelial cells produce NO which has capability to control the metastate of melanoma cell of lungs. It has been also reported that NO has the property to inhibit platelet aggregation and reduce platelet adhesion on endothelial monolayer contributing to additional effects of NO. [54]

In addition, NO has been shown to possess modulator effects on mitochondria by inactivating irreversible mitochondrial enzymes, inducing mitochondrial permeability transition and by inhibiting reversible respiration. [56] It has been reported that NO has the capability to nitosating critical thiol residue on creatinine phsphokinase which is irreversible process and it deenergizes mitochondria by disrupting ATP supply. [57] Moreover, the direct action of NO happens due to cNOS which further inhibits the mitochondrial respiration and shows its action in aerobic condition by participating with ETC to form O^{2-}. [58-59] Further, the induction of mitochondrial permeability transition is mainly done by $ONOO^-$ which oxidizes thiols and NADPH of mitochondria and consequently increasing the calcium ion efflux alongwith oxidative efflux. [60] The mitochondria decrease the membrane potential by inducing permeability ultimately leading to increased cytoplasmic Ca^{2+} ion levels. This permeability further leads to the formation of protein pores in inner membrane of mitochondria eventually resulting in the leakage of the contents. [58] Further, NO has been demonstrated to directly react with metal complexes or oxo complexes leading to the formation of metal nitrosyls. The reaction involved between NO and metal ions include the direct reaction between metals and NO, reaction of NO with O_2 metal complexes and reaction of NO with oxo complexes. NO reacts with metal ion complexes in order to form nitrosyl ion which is involved in both regulatory and cytotoxic action. [61]

NO has been well reported free radical having both cytoprotective as well as tumor suppressing properties. Various studies have comprehensively reported NO to possess various direct and indirect effects on biological systems. Moreover, numbers of physiological functions of NO have been well demonstrated in previous studies. However, sufficient amount of literature is present on NO, but new studies are warranted in order to completely understand the biochemistry of NO in order to improve the quality of life with the help of such an important agent.

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