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NITRIC OXIDE: A NOVEL THERAPEUTIC TARGET

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ABSTRACT

Nitric oxide (NO), the most potent natural vasorelaxant, has close relation to cardiovascular physiology, inspite of its complex chemistry; it is a signal-transducing radical. Aspects of NO biology critical to gastrointestinal health and, consequently, nutritional status are increasingly being recognized. Cross-talk between NO and micronutrients within and outside the gastrointestinal system affects the establishment or progression of several diseases with pressing medical needs. Some studies imply that NO has cytotoxic properties and is the genesis of numerous diseases and degenerative states, whereas other reports suggest that NO prevents injurious conditions from developing and promotes events which return tissue to homeostasis. NO or related species have been implicated in the regulation of many genes that participate in many diverse biological functions including programmed cell death or apoptosis. Along with cell apoptotic function, it also plays several roles in others parts of the body. In this review we are describing a gestalt on NO, which includes the various synthetic approaches, isoforms of NOS & its related aspects, multifactorial biological applications, its therapeutic applications in various diseases.

INTRODUCTION: NO, is the widely known biological molecule in human body. Inspite of its small size, its complex chemical nature in body provides complex physiological roles to it. NOS, one of the part of NO synthesis, play a crucial role in the synthesis of NO in the body.

At the post- translational level, NOS3 activity is highly regulated by 6 different mechanisms: lipidation, a calcium/calmodulin- dependent mechanism, direct protein-protein interactions, various phosphorylations, O-linked glycosylation, and substrate and cofactor availability. Nitric oxide (NO) free radical, inspite of having a high reactivity, is capable of mediating a multitude of reactions.

NO is a small, unstable potentially toxic gas which is highly diffusible across the cell membranes. Since its identification as the endothelium derived relaxing factor in 1987, nitric oxide (NO) has attracted a tremendous interest in a broad field of basic and applied research during the past decades.

NO is able to react with other inorganic molecules (i.e. oxygen, superoxide or transition metals), structures in DNA (pyrimidine bases), prosthetic groups (i.e. heme) or with proteins (leading to *S* -nitrosylation of thiol groups, nitration of tyrosine residues or disruption of metal-sulfide clusters such as zinc- finger domains or iron-sulfide complexes). In addition, NO can function as an anti-oxidant against reactive oxygen species (ROS) such as, hydrogen peroxide (H_2O_2) and

superoxide ($O2^-$) by diffusing and concentrating into the hydrophobic core of low-density lipoprotein (LDL). NO is involved in a variety of signaling mechanism which results in the alteration of proteins. NO has been shown to participate in a large number of pathophysiological conditions such as arthritis, atherosclerosis, cancer, diabetes, numerous degenerative neuronal diseases, stroke and myocardial infarction. NO is one of the number of positive physiologic regulators of ingestive behavior i.e., food intake, meal number, and meal duration and along with these several other functions are performed by NO.

Synthesis of NO:

Enzymatic Synthesis of NO: Nitric oxide (NO) is a highly reactive free radical capable of mediating a multitude of reactions. NO is a small, unstable potentially toxic gas which is highly diffusible across the cell membranes. The free radical, NO, is an uncharged molecule containing an unpaired electron in its outermost orbital, allowing it to undergo several reactions functioning either as a weak oxidant (electron donor) or as an anti-oxidant (electron acceptor). L-Arginine is the amino acid essential for the biosynthesis of NO as shown in **fig. 1**.

The oxidation of L-arginine is catalyzed via a family of enzymes called nitric oxide synthases (NOSs) which, in effect, yield both NO and L-citrulline using NADPH and O_2 as co-substrates. This reaction is carried out via the NOS mediated five-electron oxidation of the terminal guanidino nitrogen of L–arginine to produce NO and L-citrulline.

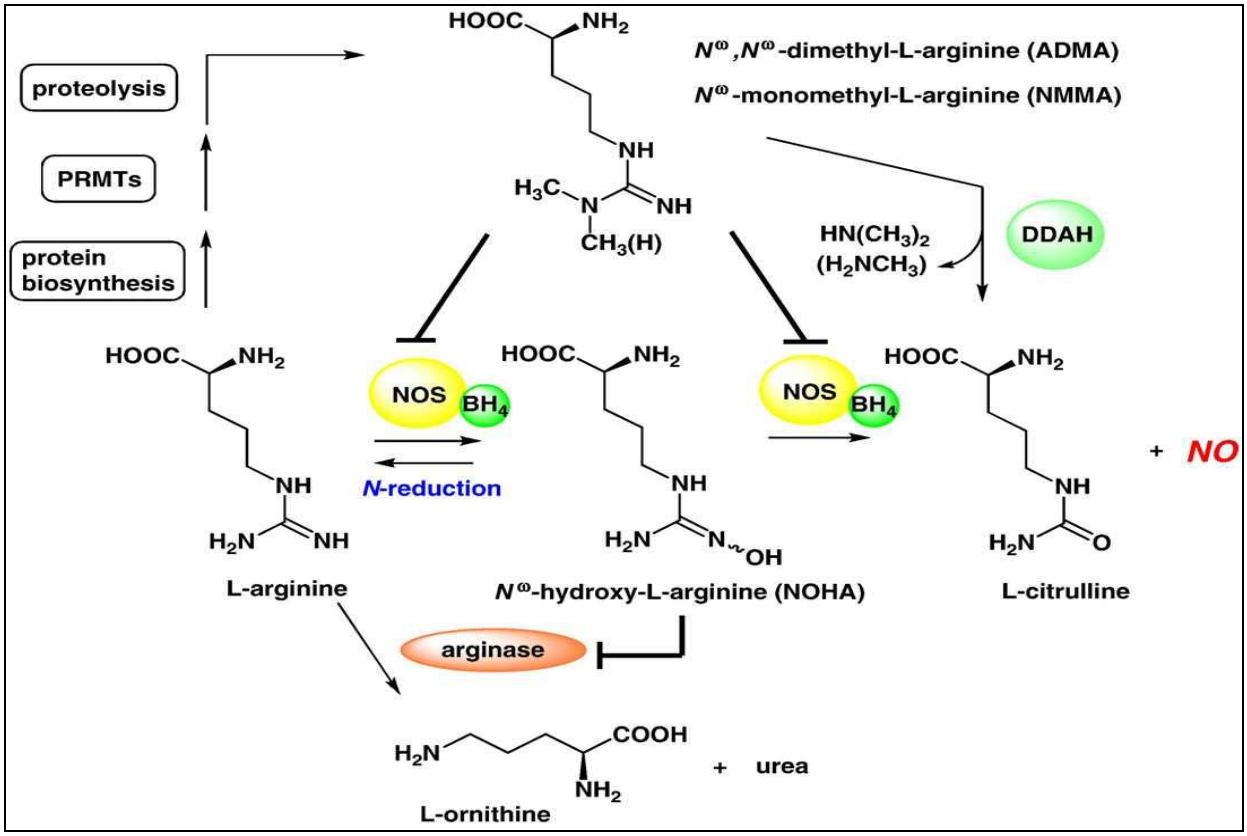

FIG. 1: SYNTHESIS OF NO FROM L-ARGININE

Non- enzymatic NO synthesis: Nitrite is an oxidation product of NO. Its plasma concentration is in the order of 0.5 µM, and its concentration in vascular tissue is as high as 10 µM [2]. Nitrite represents a major pool of NO rather than being only a biological inactive oxidation product of NO. Such a NOS-independent NO production from nitrite is accelerated under pathophysiological [3, 4] conditions as given in **fig. 2**. At low pH, as it occurs during myocardial ischemia [5-8], nitrite forms nitrous acid, which can react further with nitrite or an electron donor to form dinitrogen trioxide (N_2O_3).

This reactive nitrogen species can then nitrosate thiols or produce NO gas [9]. Alternatively, the conversion of nitrite to NO gas could be catalyzed by a metal or enzyme called xanthine oxidoreductase, which is present in abundance in vascular endothelium or nitrite can also react with deoxygenated hemoglobin to form nitrosylated hemoglobin and, to a lesser extent, S-nitrosohemoglobin (SNOHb), a reaction from which NO might be generated.

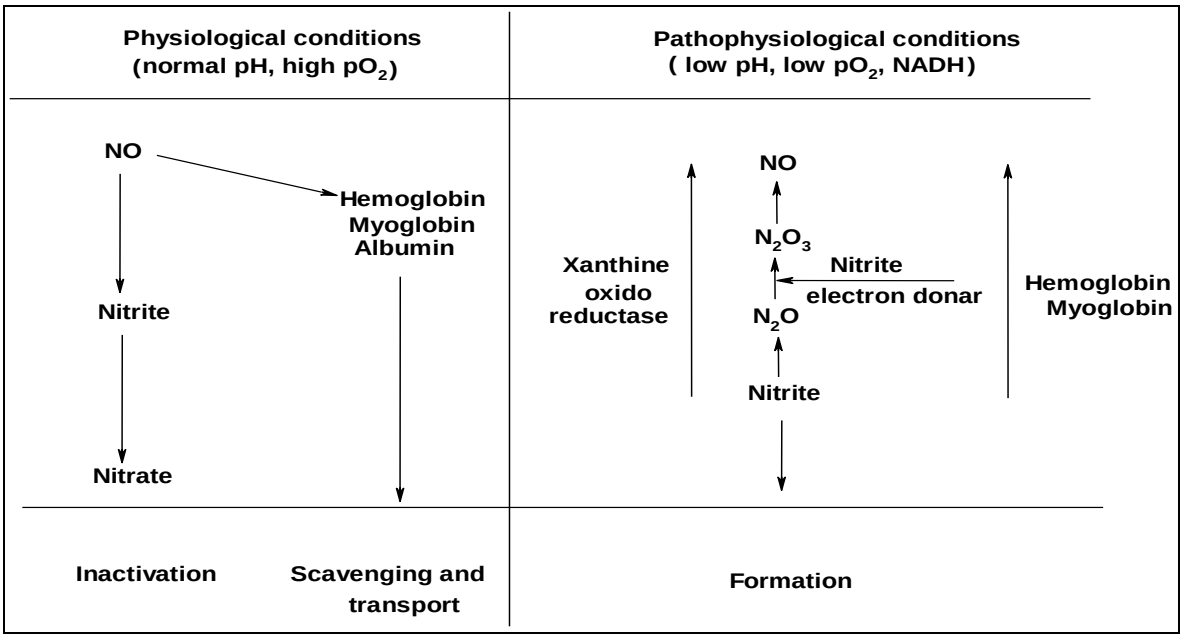

FIG. 2: NOS-INDEPENDENT NO FORMATION

Nagase *et al* [10]., demonstrated non enzymatic NO synthesis from D- arginine or L-arginine by utilizing hydrogen peroxide in the presence of L-NAME [11] (N^G-nitro-L- argininemethyl ester- NOS inhibitor). Antioxidants, mainly ascorbic acid, exert the protective action by its ability to generate NO both in a NOS-independent and dependent manner (BH_4-dependent) way.

Nitric Oxide Synthase: Nitric oxide synthase (NOS) is a dimeric enzyme that consists of two identical monomers; each monomer can be divided into two distinct catalytic domains: an NH_2-terminal oxygenase domain and a P450-like, heme containing COOH-terminal reductase domain (NOSR [12]). The latter shares a high degree of sequence homology to NADPH: Cytochrome P450 reductase (P450R [13]). The oxygenase domain is responsible for the production of NO by a two-step oxidation of L -arginine to L-citrulline in the presence of oxygen [14]. There are at least three distinct isoforms [15, 16] of the enzyme nitric oxide synthase (NOS) encoded by three different genes: endothelial, neuronal and inducible NOS genes [17] as given in **fig. 3**.

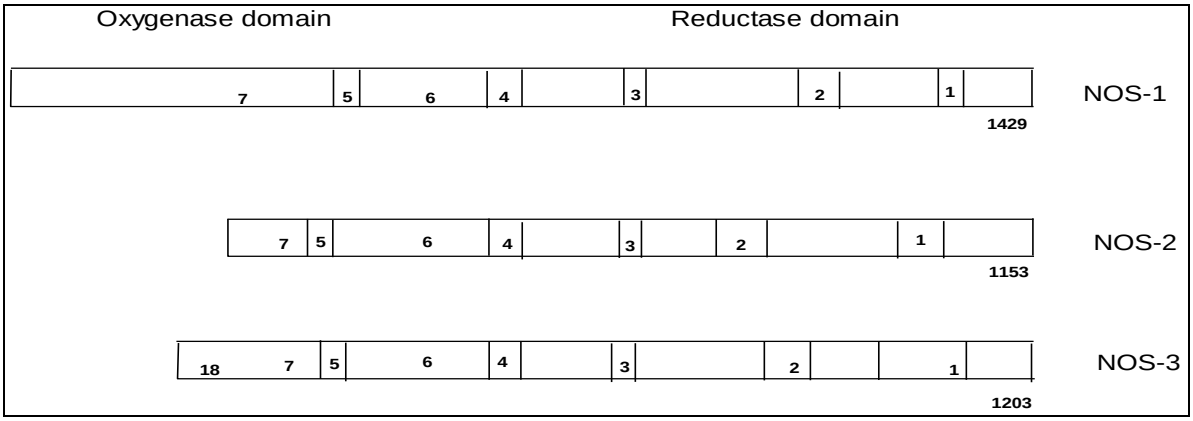

FIG. 3: THE THREE NITRIC OXIDE SYNTHASE (NOS) ISOFORMS

Numerically represented in the figure are the binding sites for NADPH (1), FAD (2), FMN (3), calmodulin (4), heme (5), arginine (6) and tetrahydrobiopterin (7). NOS 3 contain a myristoylation site at its N terminus. The number of amino acids per polypeptide chain is given for the rat enzymes [18]. The neuronal NOS (nNOS or NOS 1, 150 kDa protein, encoded by the NOS 1 gene), the inducible NOS (iNOS or NOS 2, 130 kDa protein, encoded by the NOS 2 gene), which is the only calcium independent isoform, and finally, the endothelial NOS (eNOS or NOS 3, 133 kDa protein, encoded by the NOS 3 gene). All isoforms are expressed in cardiovascular tissues. The three isoforms differ in both structure and function[19] but share about 50% sequence homology and are differentially regulated making the catalytic activity distinct for each isoform. They are expressed in a variety of cell types and isoform- specific domains direct sub-cellular localization, affecting the functional significance of the enzyme. The endothelial and neuronal types (eNOS and nNOS, respectively) are constitutive and calcium/calmodulin dependent; they are regulated primarily by calcium influx and generate low levels of nitric oxide (NO) for short periods of time as given in **fig. 4**. Both NOS I and NOS III are constitutively expressed and are important for the production and maintenance of a low basal level of NO synthesis in neural cells and endothelial cells.

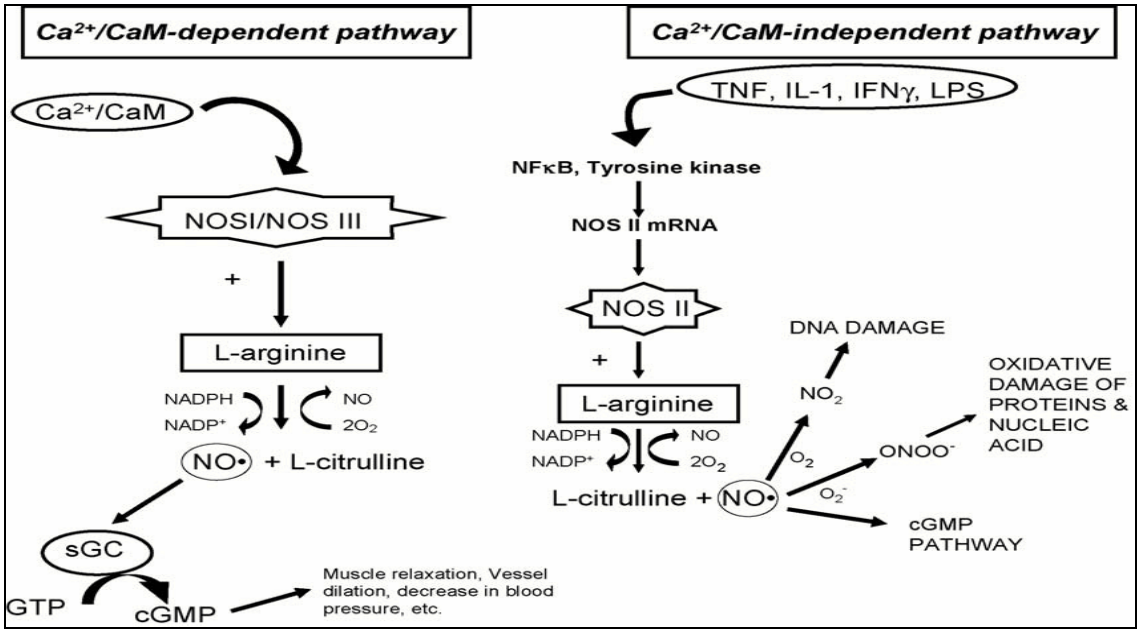

FIG. 4: SCHEMATIC REPRESENTATION OF THE TWO PREDOMINANT TYPE OF NOS ISOFORM KINETICS (CONSTITUTIVE AND INDUCIBLE) AND SUBSEQUENT NO SIGNALING [20-23]

Mechanisms involved in the regulation of Nitric Oxide Synthase Activity: At the post- translational level, NOS3 activity is highly regulated by 6 different mechanisms [24, 25;] lipidation, a calcium/calmodulin-dependent mechanism, direct protein- protein interactions, various phosphorylations, O-linked glycosylation, and substrate and cofactor availability.

Nitric Oxide Synthase Cofactors: Purified endothelial NOS has 2 bio-domains: a C- terminal reductase domain that contains nicotinamide adenine dinucleotide phosphate (NADPH), flavine adenine dinucleotide (FAD), flavine mononucleotide (FMN), and calmodulin binding sites and an N-terminal oxygenase domain that contains sites for tetrahydrobiopterin (BH4), heme, and L-arginine. The catalysis of NO synthesis at the active site requires step wise transfer of electrons initiated by NADPH binding to its site in the reductase domain. Electrons are transferred from NADPH to FAD and then to FMN, from where an electron is transferred to the heme of the oxygenase domain and converts ferric heme to ferrous that binds oxygen actively. The precise role of BH in the formation of NO still remains unclear, but it is like have an effect as an allosteric factor and/or as a redox cofactor [26].

In atherosclerosis, during ischemia/reperfusion, and/or during inflammation, the bioactivity of BH4 is reduced. Once BH4 levels are decreased, a concomitant increase of NOS-dependent generation of superoxide and subsequently peroxynitrite further reduces BH4 availability.

Indeed, free radical scavenging with vitamin C increases NOS 3 activity by increasing the availability of BH4. The most likely explanation for this may be the chemical stabilization of BH4. Without an adequate delivery of substrate and cofactors, NOS no longer produces NO but instead transfers the free electrons to oxygen and thus produces free oxygen radicals [27-30]. Free oxygen radicals, in turn, can lead to further oxidation of NOS cofactors, such as BH4, thereby aggravating the whole process [31]. Finally, there appears to be a self-inhibition of NOS in that high concentration of NO inactivates the enzyme; nitrotyrosylation of the functional NOS heterodimer causes monomerization and subsequently, inactivation of the enzyme [32].

Endogenous Nitric Oxide Synthase Inhibitors: NOS activity can be decreased by endogenous substances such as asymmetric N-methylated derivatives of l-arginine, for example, asymmetric dimethylarginine (ADMA). The intracellular ADMA concentration is controlled by dimethyl arginine dimethylamino-hydrolase [33] (DDAH). Levels of ADMA are known to correlate with certain disease states, such as the degree of atherosclerosis [34-37]. Under certain conditions when NO formation increases, S-nitrosylation diminishes DDAH activity leading to the accumulation of ADMA and, subsequently, NOS inhibition, as a type of regulatory feedback [38].

Distribution of isoforms of NOS: NOS 3, the only membrane-associated isoform, is constitutively expressed in endothelial cells, cardiac myocytes, and hippo camp a pyramidal cells and is involved in suppressing platelet aggregation, maintaining vascular tone, inhibiting smooth muscle cell proliferation and prompting angiogenesis. NOS 3 also mediates penile erection in males by diffusing NO to smooth muscle through the elevation of cGMP levels, which in turn, reduce cytoplasmic Cathus triggering relaxation of the corpora cavernosa (erectile bodies).

NOS 1, constitutively expressed in neurons, skeletal muscle and lung epithelium, is responsible for relaxation of vascular and nonvascular smooth muscle and acts as a neurotransmitter. NOS 1 is directly involved in the contraction of skeletal muscle and is responsible for producing large amounts of NO during contractile activity. The Ca2+/CaM-independent inducible isoform (NOS II/iNOS) is found in various cell types including macrophages, dendritic cells, fibroblasts, chondrocytes, osteoclasts, astrocytes, epithelial cells, and a variety of cancer cells. NOS II is stimulated and upregulated via induction by cytokines and/or microbial agents such as lipopolysaccharides (LPS) and is responsible for generating large amounts of NO sustained over long periods of time for host defense against pathogens.

Regulation of NOS 2 predominantly occurs at the level of synthesis and stability of mRNA and protein. In fact, it was observed in human cells that, although there was no detectable NOS 2 mRNA in non-induced AKN hepatocytes or DLD1 cells, significant basal activity of the human NOS 2 promoter was induced 2-5 fold by cytokines in these cells. Furthermore, human primary cardiomyocytes in cell culture were found to express NOS 2 mRNA but no NOS 2 protein. Thus, a connection can be made between the incapability of translation of NOS 2 mRNA and the presence of the 5′ or 3′ UTR of the human NOS 2 mRNA [39].

Multifaceted biological effects of Nitric Oxide [40] (NO): NO is able to react with other inorganic molecules (i.e. oxygen, superoxide or transition metals), structures in DNA (pyrimidine bases), prosthetic groups (i.e. heme) or with proteins (leading to *S* -nitrosylation of thiol groups, nitration of tyrosine residues or disruption of metal-sulfide clusters such as zinc-finger domains or iron-sulfide complexes).

In addition, NO can function as an anti-oxidant against reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide ($O2^-$) by diffusing and concentrating into the hydrophobic core of low-density lipoprotein (LDL). It can react with several ROS, such as superoxide to form peroxy nitrite ($ONOO^-$), a highly oxidizing and nitrating reactive nitrogen species (RNS) responsible for mediating protein oxidation reactions under physiological conditions.

Another mechanism of NO-related reactivity is through the addition of an NO group to the thiol side chain of cysteine residues within proteins and peptides, termed *S*-nitrosylation, which plays a significant role in the ubiquitous influence of NO on cellular signal transduction. NO or NO ion is capable of forming *S* - nitrosothiols (RSNO; product of *S*- nitrosylation), which function as potent platelet aggregation inhibitors and vasorelaxant compounds. NO is involved in a variety of signaling mechanism which results in the alteration of proteins. These modifications are concentration-dependent and include: direct binding to heme centers, nitrosylation of thiol and amine groups, nitration of tyrosine, tryptophan, amine carboxylic acid, and phenylalanine groups, oxidation of thiols, and binding of sGC and transcription factors as given in **fig. 5.**

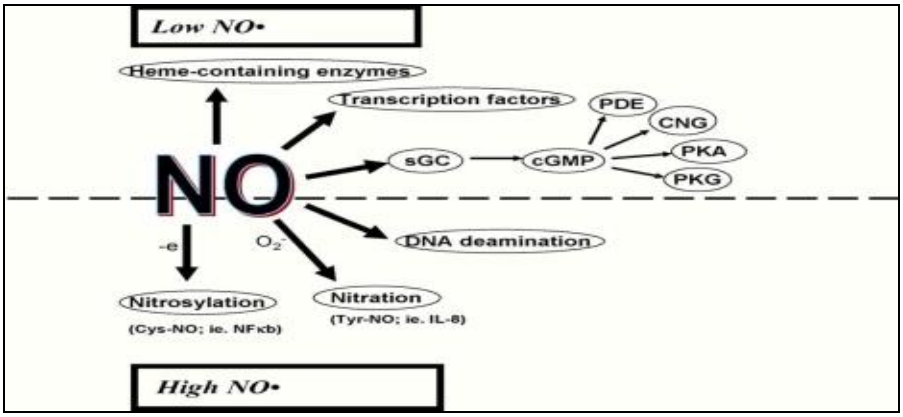

FIG. 5: NO-MEDIATED PROTEIN MODIFICATIONS

NO-mediated physiological effects are carried out primarily by 3, 5-cyclic guanylate monophosphate, more commonly known as cyclic GMP (cGMP). cGMP is a principal molecular messenger responsible for smooth muscle relaxation, platelet aggregation and neurotransmission. In its cGMP-dependent mechanism, NO binds and activates the enzyme guanylate cyclase (GC), which, in turn, catalyzes the synthesis of cGMP from guanosine triphosphate (GTP). NO is converted from arginine via NOS and binds and activates the enzyme soluble guanylate cyclase (sGC), which, in turn, catalyzes the synthesis of cGMP from guanosine triphosphate (GTP). cGMP is then converted to guanylic acid using the enzyme cGMP phosphodiesterase. This conversion allows for the appointment of a variety of downstream targets including protein kinases, phosphodiesterases and ion channels, which modify cell functions as given in **fig. 6**.

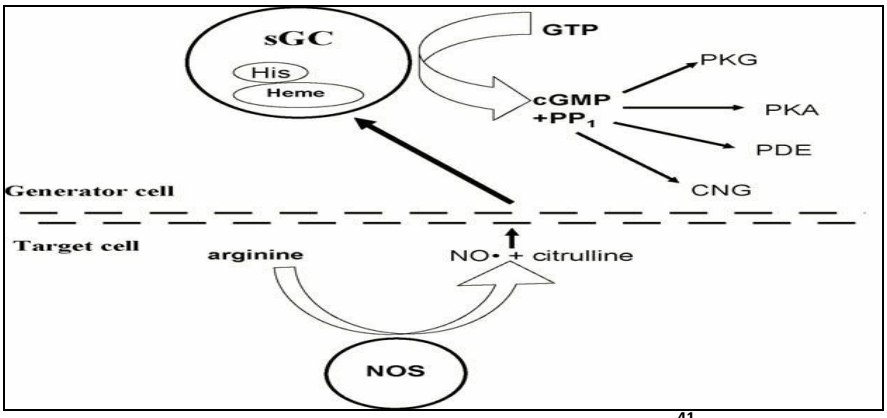

FIG. 6: CGMP-DEPENDENT CELL SIGNALING [41]

Direct and indirect effects of NO [42]: NO has been shown to participate in a large number of pathophysiological conditions such as arthritis, atherosclerosis, cancer, diabetes, numerous degenerative neuronal diseases, stroke and myocardial infarction. NO and NO-derived chemical species can inhibit enzyme function, alter DNA, and induce lipid peroxidation (Direct effects) as given in **fig. 7**.

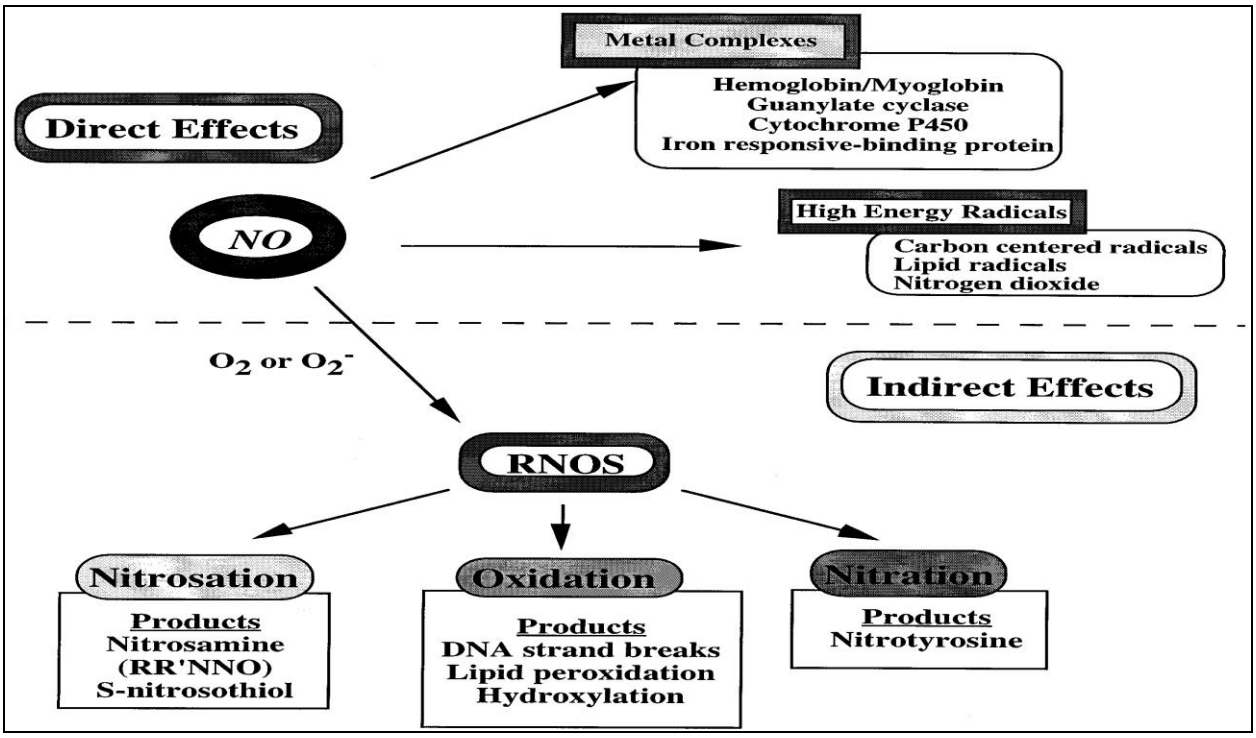

FIG. 7: DIRECT AND INDIRECT EFFECTS OF NO

NO has antioxidant properties, and the ability to protect cells against cytokine induced injury and apoptosis. NO, itself is not a powerful cytotoxic agent; however, it can render cells susceptible to other cytotoxic agents such as heavy metals, alkylating agents and radiation. Yet, NO has been shown to be protective against an array of agents that induce oxidative stress, such as hydrogen peroxide (H_2O_2), alkyl hydroperoxides and superoxide. NO reacts with some redox metal complexes, yet its reactivity varies from complex to complex.

NO reacts with other radicals such as superoxide and lipid derived radicals to form products such as $ONOO^-$ and LOONO, which can further react with other biological targets to influence different physiological and cellular functions (Indirect effects). NO can react with oxygen to form a variety of different reactive intermediates that are normally associated with smog and cigarette smoke.

These chemical species can alter critical biomolecules such as enzymes and DNA. However, NO can also neutralize oxidants associated with oxidative stress and abate reactive oxygen species (ROS)-mediated toxicity. Direct effects occur at low NO concentrations (<1 µM); whereas, indirect effects of NO involving the formation of RNOS become significant at higher local concentrations of NO (>1 µM). In some cells, iNOS is expressed after exposure to specific combinations of cytokines. Cell types containing cNOS generate low fluxes of NO for short periods of time; thus, direct effects of NO are the predominant chemistry and indirect effects are limited. However, in the presence of iNOS, production of NO is much greater and indirect effects such as nitrosation, nitration, and oxidation reactions occur.

It is clear that the source and level of NO provides a guideline as to what chemistry will most likely occur under a specific biological condition. Another important consideration is the distance of the target from the NO-generating source. Cells or tissues close to an NO source may experience both indirect and direct chemistry. Cells further away from the NO-generating source may only experience direct effects because NO concentration decreases as a result of diffusion and biological consumption.

Spatial and temporal factors are therefore important when considering the chemistry responsible for the specific biological effects.

NO as Therapeutic Agent:

NO modulates feeding behavior and helps ensure GI homeostasis: NO is one of a number of positive physiologic regulators of ingestive behavior (i.e., food intake, meal number, and meal duration [43]). Administration of a NO donor or L-arginine reduces the hypophagia caused by amylin, leptin, or a threonine free diet [44]. Endogenous NO supports normal digestive activity by ensuring GI homeostasis and defence as given in **fig. 8**. The GI tract is a major NO producer. NO protects the GI tract from chemical, drug, acid, stress, and endotoxin-induced GI injuries and facilitates the healing of gastric erosions and ulcers [45]. Exogenous NO in the GI tract can offer tissue protection as a potential therapeutic modality against toxic insult.

NO supplementation preserves GI status: Chronic NSAID use invites GI damage (irritation, erosion, bleeding, and ulceration) and overt symptoms such as dyspepsia, abdominal pain, colitis, and diarrhea. This GI liability reflects non-selective NSAID inhibition of cyclooxygenase (COX) isoforms, only one of which (COX-2) mediates inflammation, where as the other (COX-1) produces cytoprotective prostaglandins. NO supplementation attenuates NSAID gastric irritation and ulcerogenic activity and might even promote healing of preexisting lesions [46].

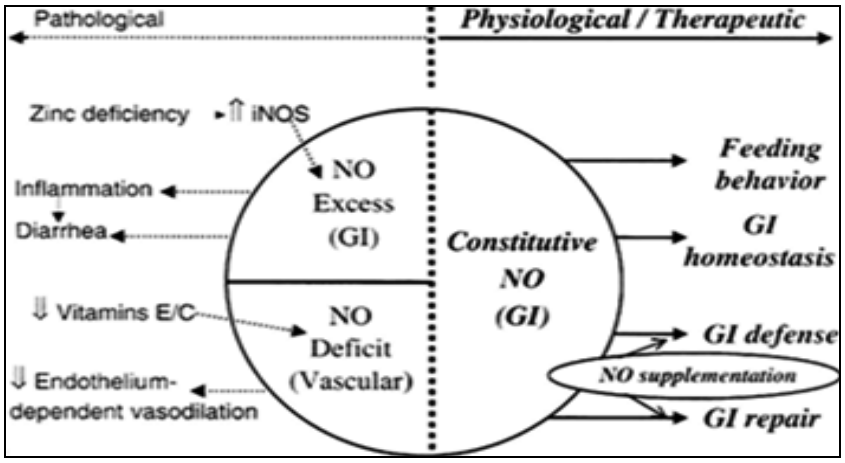

FIG. 8: SUMMARY OF PATHOLOGICAL AND NUTRITIONAL ASPECTS OF NO

Micronutrients modulate tissue NO tone in some common diseases: Among the most important micronutrients, the divalent trace element zinc is essential to the growth and development of microorganisms, plants, and animals. Zinc shows anti-inflammatory properties in vivo by direct inhibition of NOS and by suppressing the iNOS induction by cytokines [47].

Tumor radio sensitizing effects of NO: NO has intrinsic radiosensitive activity that could mimic the oxygen effect in a hypoxic environment and acts as a direct radiosensitiser [48].

NO act as a modulator of tumor immunity: Coupling a NO- releasing moiety to aspirin was shown to provide feedback inhibition of both iNOS activity and associated peroxinitrite generation, leading to a correction of immunosuppression in tumors [49].

NO donors may be categorized as 'classical' NO donors (that donate NO systemically) and 'selective' NO donors (that release NO preferentially in tumors). Examples of NO donors isosorbide dinitrate, nitroglycerin, sodium nitroprusside, molsidomine, and 2-(N, N-diethyl amino)-diazenolate-2-oxide (DEA/NO) or spermine.

NO in cancer treatment: The basal formation of NO in mitochondria by mitochondrial NOS seems to be one of the main regulators of cellular respiration [50], mitochondrial transmembrane potential and transmembrane proton gradient. NO inhibits cellular respiration in tumor cells by inhibiting cytochrome c reductase, and by redirecting oxygen from a respiratory fate to the fixation of DNA damages occurring in response to radiotherapy [51]. Delivering physiological concentration of NO to mitochondria is an attractive option for cancer treatment.

NO was primarily used for the treatment of blood vessel-related diseases and other non-cancer related applications. The demonstration of NO-mediated cytotoxicity directly on cancer cells and/or indirectly in the tumor microenvironment through its anti-proliferative and chemo-sensitizing roles, presents new challenges for its optimal use in cancer therapy.

The data suggest that NO can be used as a chemo-sensitizing as well as an immuno-sensitizing agent, and thus, one may consider its clinical application using combination treatment of NO donors and chemotherapy or immunotherapy resulting in synergistic activity in the treatment of cancer. It is also conceivable that one might use NO donors complexed with chemotherapeutic drugs or other cytotoxic agents. One may also consider using agents that can activate endogenous NO production via NOS 2. Clearly, apart from the direct effects of NO on tumor cells, NO donors would also be functioning as vasodilators and thus have an even enhanced therapeutic activity.

Hypoxia and blood flow heterogeneities are characteristics of solid tumors and are major obstacles for therapy. Exploiting the biology of nitric oxide (NO), a small radical with multiple functions, is particularly attractive to circumvent these sources of resistance and to sensitize tumor to cytotoxic treatments such as radiotherapy and chemotherapy. Indeed, while NO mediates angiogenic effects, NO may also promote tumor perfusion, drug delivery and oxygenation.

Pro- and Anti-Apoptotic capacity of NO and its use in Myocardial Ischemia and Heart Failure: It appears that high levels of NO produced by inducible nitric oxide synthase (iNOS) promote apoptosis [52] while basal levels of NO production from endothelial nitric oxide synthase (eNOS) protect cardiomyocytes from apoptosis. Since permanent loss of cardiomyocytes due to apoptosis contributes to the development of heart failure, inhibition of cardiomyocyte apoptosis may have therapeutic implications. Given its pro- and anti-apoptotic [53] capacities within the heart, NO may serve as a valuable therapeutic target in myocardial ischemia and heart failure.

NO in treatment of Cardiovascular Disorders: An impaired nitric oxide (NO) bioavailability is well-recognized in the pathology of endothelial dysfunction or atherosclerosis, respectively, and characterized by a reduced NO biosynthesis, an accelerated inactivation and/or a decreased sensitivity to NO. Therefore, attempts to increase endogenous NO concentrations or to improve responses to NO stimulation have attracted great pharmaceutical interest for the treatment of several cardiovascular diseases [54] as given in **fig. 9.** The biological system of the NO/cGMP cascade is very complex and highly regulated through diverse upstream and downstream molecular and cellular elements and feedback mechanisms.

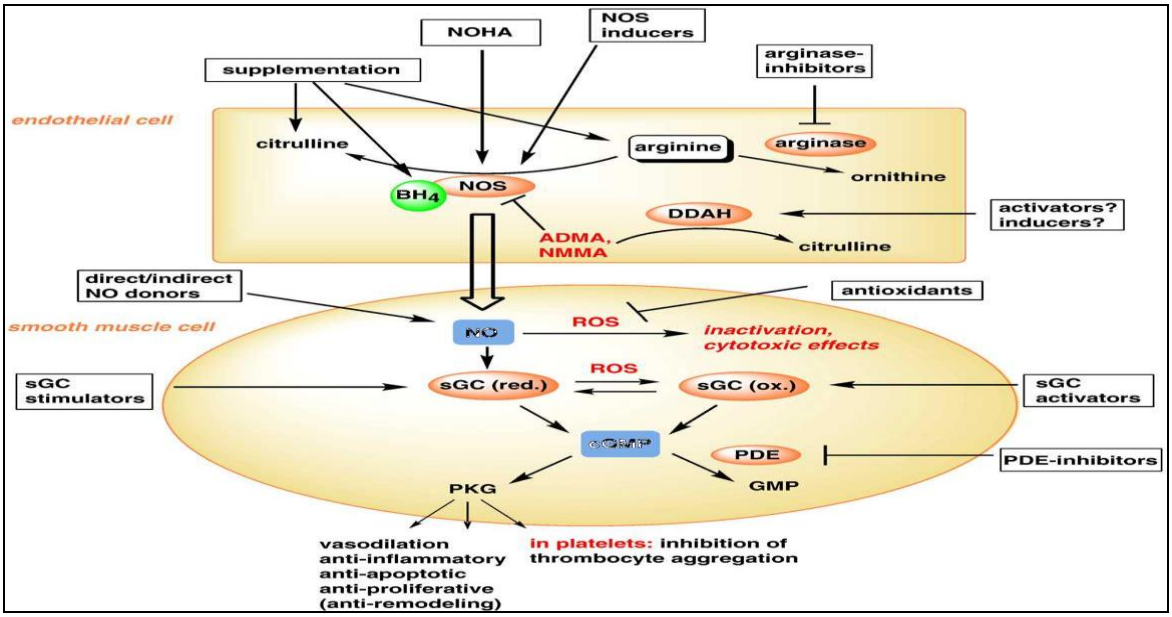

FIG. 9: OPTIONS TO MODULATE THE NO GENERATING SYSTEM IN THE VASCULATURE TO ENHANCE NO BIOAVAILABILITY

ADMA, asymmetric N^W, N^W- dimethyl-L-arginine; BH_4, tetrahydrobiopterin; cGMP, cyclic guanosine-3′, 5′-phosphate; DDAH, dimethylarginine dimethylamino hydrolase; GMP, guanosine-5′-monophosphate; NMMA, N^W-monomethyl-L- arginine; NO, nitric oxide; NOS, nitric oxide synthase; ROS, reactive oxygen species; sGC(ox),soluble guanylate cyclase (oxidized form); sGC(red),soluble guanylate cyclase (reduced form)

NO and Agmatine: Research on L-arginine was boosted after the identification of nitric oxide (NO) and agmatine and their physiological importance. NO directly modulates ion channels, activates soluble guanylate cyclase and other important proteins by ADP ribosylation and nitrosylation and binding to heme or iron–sulfur clusters. These modifications and interaction with heme might activate or inhibit various protein kinases, phosphatases and modulate transcription of various nuclear factors to possibly cause cardiovascular diseases like hypertension, ischemia, diabetes, atherosclerosis and angiogenesis. Agmatine holds the key to prevent the toxic effects associated with induction of NO synthesis by its ability to inhibit inducible nitric oxide synthase [55, 56] (iNOS).

Agmatine is also synthesized from L-arginine by the enzyme arginine decarboxylase and displays a significant potential in cardiovascular system. Agmatine [57], with the myriad of effects on calcium homeostasis, seems to modulate various functions in the heart, brain and vasculature.

NO regulates HIF-1 activation and Angiogenesis: HIF-1(Hypoxia Inducible Factor 1)-mediated angiogenesis is controlled by PHD2, an oxygen sensor prolyl hydroxylase that normally addresses HIF-1α for proteasomal degradation in normoxic cells. Redox transfer of two hydroxyl residues on the oxygen-dependent domain of HIF-1 α (dashed/deep blue) requires O_2 and 2-oxoglutarate as substrates and Fe^{+2} at the catalytic site of the enzyme as given in **fig. 10**.

Hydroxylated HIF-1 α (on Pro402 and Pro564), succinate and CO_2 are products of the reaction, whereas the oxidized iron is further reduced by ascorbate for enzyme recycling. The low affinity of PHD2 for oxygen couples local pO2 to HIF-1 α stabilization and HIF-1 activity: at high pO_2, hydroxylated HIF-1 α is addressed for degradation through the von Hippel-Lindau (pVHL)-proteasome pathway; at low pO_2, however, HIF-1 is stabilized and transcriptionally activated to promote VEGF expression and angiogenesis (the inserted microphotograph shows endothelial cells networking from an explanted tumour microvessel cultured on a Matrigel matrix). Coupling pO_2 and angiogenesis is further regulated by factor inhibiting HIF-1 (FIH-1), an oxygen- and 2-oxoglutarate-dependent asparagine hydroxylase that inactivates HIF-1 α under normoxia.

NO influences this pathway at several levels. Under normoxia, NO may act as a hypoxiamimetic through;

1. Inactivating the catalytic Fe^{+2} site of PHD2,
2. Inactivating FIH-1 probably via S-nitrosylation,
3. Directly S nitrosylating HIF-1a on Cys520, which prevents ubiquitylation and,
4. Inhibiting mitochondrial respiration at the cytochrome c oxidase (Cyt. c ox.) to elevate free O_2.
5. It also acts as a downstream pro-angiogenic effector of VEGF in ECs.
6. Conversely, NO may repress angiogenesis under conditions of oxidative stress, most probably because the high reactivity of NO for ROS neutralizes the PHD2-inactivating activity of both species.

Normal NO production in the lungs regulates basic airway events such as modification of airway tone, regulation of pulmonary vascular tone and stimulation of mucous secretion via its binding to sGC and the subsequent formation of cGmp. NO have been found to play a significant role in variety of diseases of the immune system including chronic asthma, rheumatoid arthritis & autoimmunity.

NO generated by iNOS has been shown to suppress viral replication and infection. NO protects neurons from apoptosis via S-nitrosylation and inactivation of NMDA (N-methyl-D-aspartate) receptors. NO also regulates Ca^{+2} channel activity. NO by inhibiting L-type Calcium channels protect cardiomyocytes from apoptosis.

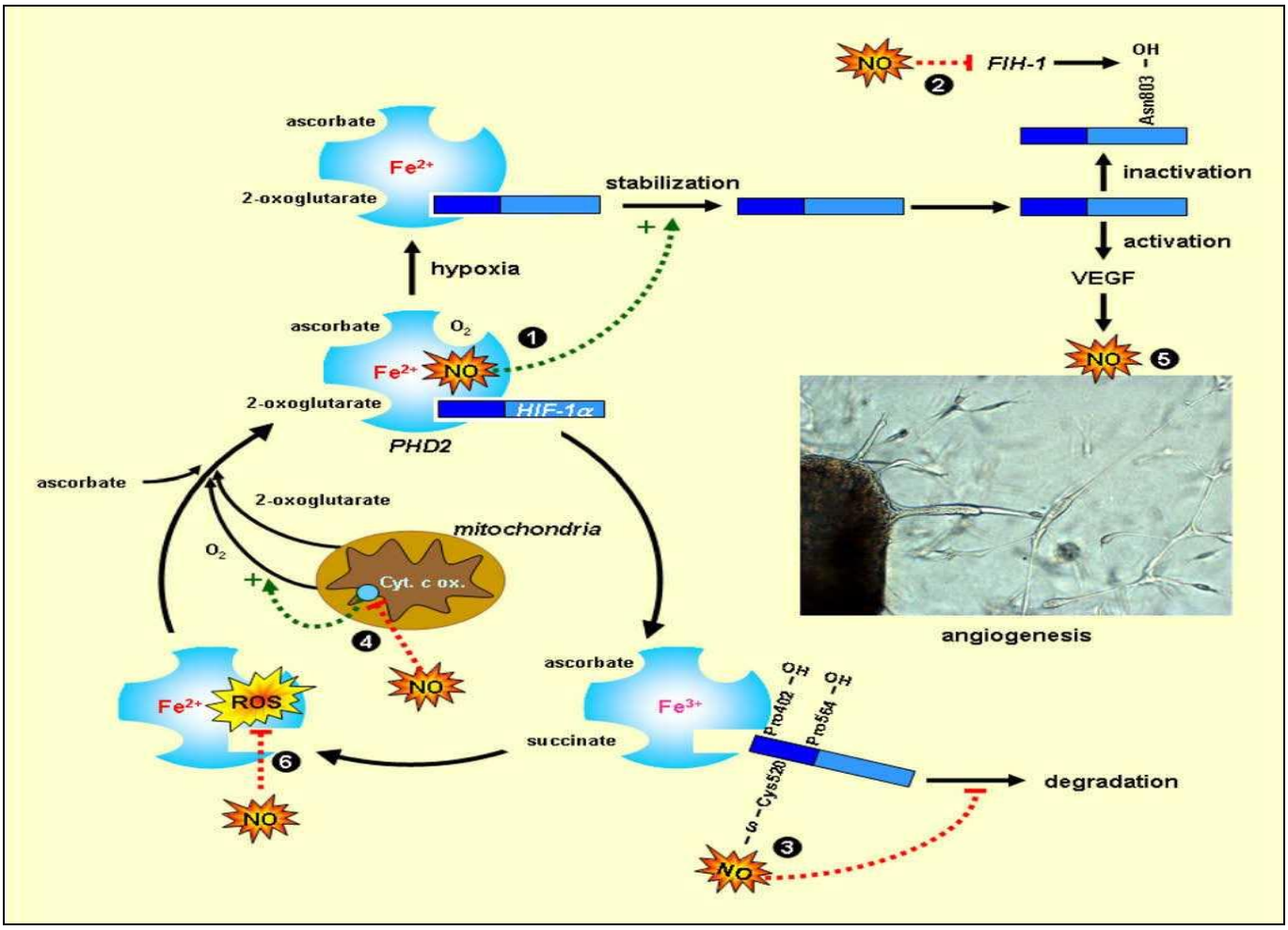

FIG. 10: ROLE OF NO IN ACTIVATION OF HIF-1 AND ANGIOGENESIS

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