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The Role of Nitric Oxide in Health and Diseases

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Context: Nitric oxide (NO) is a free radical and synthesized by three Nitric Oxide isoforms. NO is a biological messenger with many important effects on variety of biological processes. NO at low concentrations is beneficial, but has harmful effects on body health in high levels. The purpose of this review was to investigate the role of NO in health and diseases.
Evidence Acquisitions: We conducted a literature review on published English articles using databases from PubMed, SCOPUS, Google Scholar, and IranMedex between 1984 and 2013. First, 798 articles were identified; 92 articles were finally included in this literature review based on the inclusion criteria. There was an important association between serum/plasma NO levels and cardiovascular disease, hypercholesterolemia, heart failure, myocarditis, myocardial ischemia, atherosclerosis, hypertension, diabetes and dysglycemia, thyroid disorders, metabolic syndrome and obesity. Furthermore, NO has a strong trace in neurodegenerative diseases.
Conclusions: All three NOS isoforms play important roles as dichotomous effects in human biology and diseases. NO has a very short half-life, so its direct determination has limitations. The most common method for NO determination is based on the Griess reaction and NO metabolites determination.

Keywords: Nitric Oxide; Diabetes Mellitus; Obesity; Hypertension; Cardiovascular Systems; Neurodegenerative Diseases; Atherosclerosis

1. Context

Nitric oxide (NO) as a biological mediator plays an important role in a variety of biological processes and is a fundamental component in the fields of biochemistry, physiology, immunology and neuroscience (1, 2). NO is synthesized as a free radical from L-arginine by the enzyme Nitric Oxide synthase (NOS) (Figure 1), which is encoded by separate genes and is one of the most regulated enzymes in biology (2). Several cofactors have been found to be involved in this process including oxygen, tetrahydrobiopterin (BH_4), nicotinamide-adeninedinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) (3, 4).

There are three identified NOS isoforms (Table 1); neuronal (nNOS or NOS_1) and endothelial (eNOS or NOS_3 or cNOS) isoforms are constitutive (cNOS) and calcium-dependent; whereas, the last one is inducible (iNOS or NOS_2) and Ca^{2+} independent (4-6). NO as a retrograde neurotransmitter is produced in the central and peripheral nervous systems by the neuronal isoform. Therefore, it is probably important in memory and learning. Other physiological functions of nNOS include regulation of cardiac function and peristalsis and sexual arousal in males and females. An alternatively spliced form of nNOS is a major muscle protein that produces signal in response to calcium release from the sarcoplasmic reticulum (SR) (5, 7). The inducible isoform iNOS generates large amounts of NO as a defensive mechanism. It is synthesized by many cell types in response to cytokines and is an important factor in the response of body to attack parasites, bacterial infection and tumor growth. It is also the cause of septic shock and may play a role in many diseases with an autoimmune etiology (4, 5). Endothelial NOS produces NO in blood vessels and plays role in regulation of vascular function, cardiac function, angiogenesis, insulin secretion and airway tone. eNOS is the primary controller of smooth muscle tone (5, 6).

Non-enzymatic NO production by one electron reduction of nitrite, a blood and tissue NO reservoir, seems to be ubiquitous and greatly accelerated under hypoxic conditions. This finding changes the general beliefs that nitrate and nitrite are waste products of NO (8, 9). NO has the potential to react with oxygen, metals and free radicals (3, 4). Biological reactions of NO forms (NOx) have been divided into NO_2, N_2O_3, NO_2 (nitrite) and NO_3 (nitrate) in blood, which depend on diffusion, convection, distribution coefficient and chemical reactions within different compartments of human blood NO metabolites (no metabolism, no hormone) (1, 3, 4). Rates

of formation and clearance of NO determine its steady state concentration (3).

NO at low concentrations (nano molar) is beneficial, but can be toxic in higher levels (micro molar) or in the presence of Reactive oxygen species (ROS) (H_2O_2, $O_2^{\cdot\,-}$) (4). Nowadays, an association has been found between serum/plasma NO levels and different diseases including cardiovascular disease (4, 10, 11), heart failure (4, 12), hypertension (4, 13, 14), diabetes and dysglycemia (15-17), thyroid disorders (18), metabolic syndrome (17, 19, 20) and obesity (21, 22) (Table 2). In addition, it has been shown that excessive NO production impairs β cell function causing death (23); while inducible NOS is involved in muscle insulin resistance (24) lack of eNOS causes insulin resistance (25). All three isoforms of NOS are targets for thyroid hormones (26). In human hyperthyroidism, increased NO production plays a role in vasodilation and abnormal vascular tone (27). Rodriguez-Arnao et al. showed decreased NO production in newborns with primary congenital hypothyroidism (28). The aim of this study was to investigate physiological and pathological role of NO and related diseases.
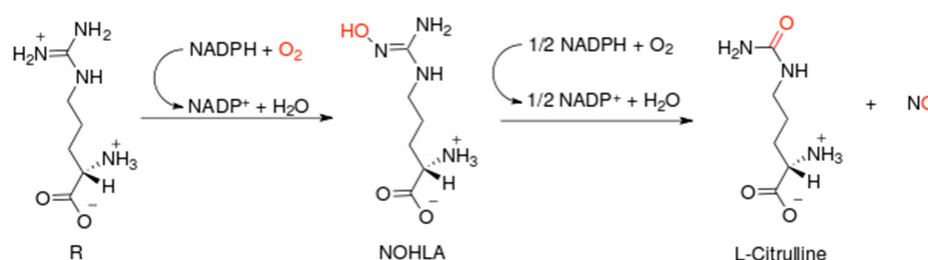
Table 1. Characteristics of NOS Isoforms

Name	Gene(s)	Location	Function	Ca^{2+} Dependency
Neuronal NOS (nNOS, NOS_1)	NOS_1, chromosome 12	nervous tissue, skeletal muscle type II	cell communication, signal transduction, neurotransmission	Ca^{2+} dependent
Inducible NOS (iNOS, NOS_2)	NOS_2, chromosome 17	Immune system, cardiovascular system	Immune defense against pathogens	Ca^{2+} independent
Endothelial NOS, (eNOS, NOS_3, cNOS)	NOS_3, chromosome 7	endothelium	vasodilation, modulation of platelet aggregation, modulation of leukocyte endothelial interactions	Ca_{2+} dependent

Table 2. NO Isoforms in Some Diseases

Disease	NO Isoform	Reference
Cardiac diseases	↑iNOS	(4, 5, 10, 29-32)
Myocardial ischemia and reperfusion injury		(4, 33)
Myocarditis		(4, 29, 34-37)
Heart failure		(4, 29, 38-43)
Vascular diseases		
Atherosclerosis	↑eNOS	(4, 5, 10, 27, 44)
Hypertension	↑eNOS	(4, 13, 45)
Aging	↓eNOS	
Neurodegenerative disorders	↑iNOS	(46-48)
Parkinson's disease		
Alzheimer's disease		
Huntington's disease		
Multiple sclerosis (MS)		
Amyotrophic lateral sclerosis (ALS)		
Traumatic brain injury (TBI)		
Local inflammation	↑iNOS	(4, 49-52)
Chronic arthritis		
Inflammatory bowel diseases		
Tissue inflammation from toxic origin		
Cancer	↑iNOS, eNOS, nNOS	(4, 53, 54)
Metabolic syndrome	↑iNOS	(17, 19, 21, 55-58)
Diabetes	↑eNOS	(4, 13, 59, 60)
Obesity	↑iNOS	(60-63)
Dyslipidemias (particularly hypercholesterolemia and hypertriglyceridemia)	↑eNOS	(64-67)

Figure 1. L-Arginine (R) is Converted to Nω-Hydroxy-L-Arginine (NOHLA) as an Intermediate

L-arginine (R) is converted to Nω-hydroxy-L-arginine (NOHLA) as an intermediate in a reaction requiring one O_2 and one NADPH and the presence of tetrahydrobiopterin (BH_4), flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), and haem; in the second step, by oxidation of Nω-hydroxy-L-arginine (NOHLA), Citrulline and NO are formed (47).

2. Evidence Acquisitions

The study methodology was searching the available literature in MEDLINE (using PubMed), EMBASE, Web of Science and Internet and compilation of all published studies from 1984 to 2013. We included any cross-sectional, longitudinal or review article in English reporting the role of nitric oxide in diseases and health. More than 798 studies were reviewed. A total of 706 articles were excluded after title and abstract assessment. Finally, 92 were included in this literature review. Used keywords were "nitric oxide", "cancer", "diabetes", "obesity", "hypertension", "cardiovascular", "neurodegenerative" and "atherosclerosis".

3. Results

3.1. Mechanism of NO

There are several mechanisms of biological activity of NO, such as oxidation of iron-containing proteins, activation of soluble guanylate cyclase, ADP ribosylation of proteins, protein sulfhydryl group nitrosylation and iron regulatory factor activation (68, 69). NO is demonstrated to activate NF-κB in peripheral blood mononuclear cells, an important transcription factor in iNOS gene expression in response to inflammation (5, 70). NO acts through the activation of soluble guanylate cyclase (cGMC). Cyclic-GMP activates protein kinase G, which causes reuptake of Ca^{2+} and opening of calcium-activated potassium channels. Decreased Ca^{2+} concentration leads to relaxation of smooth muscle cells (4, 5, 10).

Biological activity of NO inhibitors include increased reactive oxygen species (ROS), decreased L-arginine uptake, decreased cofactors (Ca^{2+}, calmodulin, BH_4), inhibition of NOS expression, inhibition of electron flow (nicotinamide adenine dinucleotide phosphate-oxidase (NADPH), flavins), inhibition of substrate binding to NOS and NO scavengers. Excessive ROS reacts with NO to pro-

duce potently toxic peroxynitrite, which contributes to vascular oxidative stress (11, 38). Many studies reported that pharmacologic effective inhibitors of all types of NOS such as NG-nitro-l-arginine methyl ester (L-NAME) produced an inhibitory action on long-term potentiation and significantly reduced the pathophysiological effects of NO in diseases due to uncontrolled production of NO (4, 71, 72).

3.2. Nitric Oxide and its Metabolites Determination Method

Measurements of NO concentration in biological samples need careful considerations. NO half-life is very short (less than one second) in biological matrix (73, 74). Nitric oxide is quickly oxidized to nitrite and nitrate as NO metabolites in plasma and in whole blood respectively by oxygen (3). The major breakdown and main stable end product of NO in the body is nitrite (3, 74); while is nitrate in the presence of sufficient amount of O2 (3).

The half-life of nitrate and nitrites in circulation are 5-8 hours and 110 seconds, respectively (73). Determination of NO itself is difficult due to its very short half-life; hence nitrite and nitrate are most often measured as a backup for NO production (75, 76). It has been suggested that measurement of serum/plasma NO metabolites are required to evaluate endothelial dysfunction (4). Since a strong correlation has been established between endogenous NO production and serum NO levels (17, 77), determination of these inorganic NO metabolites in the circulation seems to be the most suitable method of NO production quantification (78) and has been considered as an index of generalized NO production (55). Reference values for serum NO concentration have been reported in both adults (21) and pediatrics (56).

Therefore, measuring nitrite and nitrate concentrations in biological sample, notably plasma, serum and urine, is the most suitable method to assess NO synthesis in vivo (10, 74-76). Although there are various meth-

ods for determination of NO including UV absorbance measurement, GC-MS, HPLC, ion-selective electrodes and capillary electrophoresis, fluorescent assays, chemiluminescence assay and electrochemical detection (73, 74, 79, 80), the simplicity, rapidity and cost-effectiveness of the Griess assay have made it more popular than others (73, 74). The Griess assay was first described by Johan Peter Griess in 1879 (74, 78). NO measured with Griess reaction has shown a good correlation with (Gas chromatography mass spectrometry (GC-MS)) method, which is the most accurate quantitative method of NOx determination in serum (78).

Nitrite can be measured directly, without any derivatization step, or indirectly after a derivatization step such as the Griess reaction (81). Direct measurement of NO from human vessels has been performed, with extreme difficulty, and it is unlikely that NO can be measured quantitatively in vivo (82, 83). The short half-life of NO and its instability has precluded its direct measurement (82) and therefore indirect measurement of NO including accumulation of NO, S-nitrosothiols, or the increase in cGMP levels are commonly performed as an index of NO production (84). In the Griess assay, nitrite reacts with sulfanilic acid at low pH (diazotization) to form the diazonium ion, which then couples to α-naphthylamine to form a red violet colored (purple) azodye which can be monitored spectrophotometrically at 540 nm (73, 74). Different variants of the Griess reaction are available (74, 84). The modified Griess reaction using sulfanilamide and N-(1 naphtyl) ethylenediamine dihydrochloride (NEDD) is currently the most frequently used method (74).

However, accurate determination of plasma/serum concentrations of nitrate/nitrite using the Griess assay is subjected to interferences and can be influenced by several factors including: 1) contribution from other nitrate sources such as diet or drugs (e.g. nitroglycerin, isosorbide dinitrate or angiotensin converting enzyme inhibitors) and nitrate formation in saliva and bowel (3); 2) type of anticoagulant used for plasma preparation (85); 3) reduction of nitrate to nitrite (86); 4) removal of proteins (73); 5) nitrite/nitrate contamination (78); and 6) altered renal function and extracellular fluid volume (3).

3.3. Nitric Oxide and Cardiovascular Diseases

3.3.1. Cardiovascular Diseases (CVDs)

Cardiovascular diseases (CVDs) are the number one cause of deaths worldwide. More people die annually from CVDs than any other cause (29, 87). CVDs are a group of diseases of heart, the blood vessels or both (Table 2). Cardiovascular diseases refer to any disease affecting the cardiovascular system, principally cardiac disease, vascular diseases of the brain and kidney and peripheral arterial disease. The cause of cardiovascular disease is diverse, but atherosclerosis and hypertension are the most common ones. Additionally, several physiological and morphological changes occur with aging that alter cardiovascular function and lead to subsequently increased risk of cardiovascular disease, even in healthy asymptomatic individuals (4, 10, 29).

NO is synthetized in cardiomyocytes, endocardial endothelium, coronary endothelium and cardiac nerves by eNOS and nNOS which are Ca^{2+}-dependent (4). NO as an endothelium-derived relaxing factor (EDRF) plays many important roles in physiological regulation of cardiac function including coronary vasodilation, activation and modulation of cardiac contractile function, inhibiting platelet, neutrophil adhesion and inhibiting cardiac oxygen consumption (4, 5, 10, 30). However, high levels (μM) of NO and injuries or dysfunctional endothelial cells with a loss of endothelium-derived nitric oxide (NO) have been related to cardiovascular diseases (4, 5, 31). Cardiovascular diseases are associated with an increased production of ROS in the vessel wall by NADPH oxidases, enzymes of the mitochondrial respiratory chain and uncoupled eNOS. Produced isoforms of ROS ($O_2^{.-}$) in vascular wall is expressed in smooth muscle cells and endothelial, as well as the adventitia (4, 5).

Pacher et al. reported that Peroxynitrite may have an important role in myocardial and vascular dysfunction during ischemia and reperfusion (I/R), myocarditis, chronic heart failure and various other cardiovascular pathologies when expose to large amounts of peroxynitrite due to iNOS overexpression, increased superoxide production and loss of ATP-dependent K^+ channel function (4). In addition, Furchgott et al. showed that acetylcholine acts as a relaxation agent of blood vessels if the endothelium is undamaged (31). NO as an endothelium-derived relaxant factor is responsible for acetylcholine-stimulated relaxation. NO as an endogenous nitro-vasodilator mediates local regulation of basal arterial tone and is anticipated to vary in certain pathologic states (4, 10). The protective role of NO in ischemic heart include decreased intracellular Ca^{2+} concentrations through activation of cyclic guanosine monophosphate (cGMP) dependent protein kinase by activation of smooth muscle soluble guanylyl cyclase (4, 32).

3.3.2. Heart Failure (HF)

Heart failure (HF) is a chronic and progressive condition in which the heart muscle is unable to provide a sufficient pump action to maintain blood flow through meeting the body's need for blood and oxygen. Basically, the heart cannot keep up with its workload (4, 38). Common causes of heart failure are myocardial infarction and other forms of ischemic heart disease, hypertension and cardiomyopathy (29).

Many experimental and human studies have shown that various forms of HF are associated with excessive activity of iNOS in the myocardium of both animals and patients and benefits of iNOS inhibition on cardiac function; the role of iNOS and NO in the development and progression of heart failure is a controversial issue (4, 39,

40). An important component of HF is loss of peripheral and coronary vascular eNOS activity. Decreased eNOS and complex endothelial dysfunction lead to decreased NO bioavailability attributable to increased oxidant stress as well as agonist-specific receptor defects (41). Gealekman et al. found depressed myocardial contractility and beta-adrenergic hyporesponsiveness in rats due to increased myocardial iNOS activity (42). In contrast, Jones showed that iNOS deficiency did not reduce HF in mice (43). In heart failure, deregulation of cGMP signaling pathway is most often due to reduced production of NO and its increased degradation with superoxide (4).

3.3.3. Myocarditis

Myocarditis or inflammatory cardiomyopathy is inflammation of heart muscle (myocardium). Myocarditis is often due to viral infection, autoimmune reaction or as a hypersensitivity response to drugs (34). The definition of myocarditis varies, but the central feature is an infection of the heart, with an inflammatory infiltrate and damage to the heart muscle, without blockage of coronary arteries defined as a heart attack (myocardial infarction) or other common noninfectious causes (29).

NO has a debatable role in myocarditis yet. Some studies showed a beneficial excessive iNOS in mice heart due to viral Coxsackie B3 myocarditis (4, 35), however in another study, mice heart infection was associated with increased iNOS expression, increased number of heart lesions and increased myocardial nitrotyrosine accumulation (4). In addition, increased nitrotyrosine formation and iNOS overexpression were reported in human coronary arteries of patients with human transplant coronary artery disease and during cardiac allograft rejection both in experimental models and human hearts (4, 36, 37).

3.3.4. Myocardial Ischemia/Reperfusion (I/R) Injury

Myocardial ischemia/reperfusion (I/R) injury may occur in tissue injury in hypoxic conditions such as cardiopulmonary bypass, myocardial infarction and stroke. Myocardial ischemia occurs when the blood flow to the heart (coronary arteries) is decreased. This reduction in blood flow decreases heart's oxygen supply (4, 33). In tissue damage conditions, release of NO from the endothelium is beneficial during I/R effects by influencing oxygen consumption, leukocyte adhesion, platelet aggregation and free radical scavenging (4). In contrast, high concentrations of NO as a result of increased iNOS expression may potentiate ROS-mediated toxicity by promoting the formation of highly reactive species, such as peroxynitrite (4, 33).

3.3.5. Hypertension

Hypertension is occasionally called arterial hypertension. Low-grade inflammation in vascular tissue is an important contributor to the pathophysiology of hypertension, initiation and progression of atherosclerosis and development of CVD. Uncontrolled high blood pressure increases the risk of myocardial infarction, coronary arterial disease and cardiac and renal failure (4, 10, 88). Specifically, NO acts as a smooth muscle relaxation signal to generate vasodilation by activation of guanylate cyclase pathway. The communication between endothelial cell and smooth muscle cell is very important in this process. Endothelial dysfunction promotes atherosclerotic plaques by inducing hypertrophy, inflammation, thrombosis and vasoconstriction in smooth muscle cells (4, 45).

Interestingly, only effect of excessive superoxide production is not enough to generate endothelial dysfunction; just concurrently increased eNOS expression and superoxide production may cause abnormal endothelial function related to increased formation of nitrotyrosine (10, 45). Many experimental studies reported that hypertension is associated with endothelial dysfunction and excessive eNOS and superoxide production (4, 10, 13, 38, 45).

3.3.6. Atherosclerosis

Atherosclerosis is a chronic inflammatory response in arteries wall causing artery wall thickness due to endothelial injury that leads to accumulation of fatty materials such as cholesterol, triglyceride, macrophages, platelet aggregation and leucocyte adhesion (4, 44). The main critical causes in the pathogenesis of vascular diseases are endothelial dysfunction and reduced production or bioactivity of NO (10). However, many experimental human and animal studies showed an increase rather than a decrease in eNOS expression in atherosclerosis and diabetes (4, 5).

The role of eNOS in vessel function is very important, thus enhanced NO production may be important in initiation, progression and clinical expression of atherosclerosis (44). In atherosclerotic lesions, both iNOS and nNOS are expressed in vascular smooth muscle cells as a response to tissue injury, and iNOS is also expressed in activated monocytes and macrophages (4, 5). Pacher indicated that many studies reported an increase in peroxynitrite formation and superoxide in hypercholesterolemia, hyperlipidemia and hyperhomocysteinemia, which can lead to atherosclerosis (4). In addition, Bian et al. demonstrated that oxidative modification of low-density lipoprotein (LDL) by peroxynitrite is an important initial event of atherosclerosis (10).

3.4. Nitric Oxide and Neurodegenerative Diseases

Human neurodegenerative diseases are all characterized by untimely death of brain cells, including Huntington's disease (HD), Multiple sclerosis (MS), Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD) and cerebellar degeneration. Depending on its local concentration and duration of release, NO can contribute to either protective or cell destructive actions on neurons (46).

NO as a chemical messenger can quickly diffuse across cell membranes, in contrast to conventional neurotransmitters, which cannot pass cell membranes and mostly localized in the central nervous system, where may play important roles in neurodevelopment, neurotransmitter release and reuptake, synaptic plasticity, and contribute to learning and memory mechanisms (Figure 2). However, excessive production of NO alone or in combination with superoxide anion and formation of peroxynitrite can lead to neurotoxicity and neurodegenerative diseases by inhibition of mitochondrial respiratory chain components, (46, 47). Nitric oxide is associated with elevated level of iNOS activity and NO production in pathological conditions (46). Heneka reported that expression of iNOS increased in glial cells and neurons during Alzheimer's disease (AD) and other neurodegenerative conditions, which lead to apoptosis in primary neurons (48). Peroxynitrite, a powerful oxidant, can react with aromatic amino acid residues such as tyrosine to form nitrotyrosine (46). Calabrese et al. showed nitrotyrosine as a marker of nitrosative stress in patients with Alzheimer's and Parkinson's diseases (47).

3.5. Nitric Oxide and Inflammatory Diseases

Inflammatory diseases, including rheumatoid arthritis, asthma, atherosclerosis, bowel diseases and endotoxin-induced multiple organ injury are generally associated with release of pro-inflammatory cytokines, as well as inflammatory mediators such as NO (4, 49, 89, 90). NO production plays an important role in pathological and physiological conditions as pro-inflammatory (immunostimulatory, antiapoptotic) and anti-inflammatory (immunosuppressive, pro-apoptotic), respectively (50, 51). Under pathological conditions, iNOS produces excessive NO, which interacts with superoxide anion to form peroxynitrite that helps to perpetuate tissue injury and inflammatory response (4, 52). Therefore, iNOS selective inhibitors and NOS nonselective inhibitors are beneficial for the treatment of NO-induced inflammation (50).

3.6. Nitric Oxide and Cancer

The role of nitric oxide in carcinogenesis is so controversial and complex. NO has been actually demonstrated to be both tumoricidal and tumorigenic, depending on the concentration, location and timing (Figure 3) (53). NO affects all three different stages of carcinogenesis including initiation, promotion and progression by modulating cell division and growth, apoptosis, inflammation, angiogenesis and metastasis (53, 54). Mechanisms of pro-tumorigenic effects of NO include genotoxicity and mutagenicity that peroxynitrite may be responsible for DNA mutations as a main factor in the procarcinogenic relating NO overproduction (53). Overexpression of iNOS as a pro-neoplastic produces high level of NO in many human cancers. eNOS and nNOS have high expressions in some cancers such as lung, breast and brain (4). Although, NO plays an important role as an antitumor in human biology and cancer treatment. The therapeutic potential of NO as a cytotoxic agent is used in chemotherapy, radiotherapy and immunotherapy (4, 53, 54).
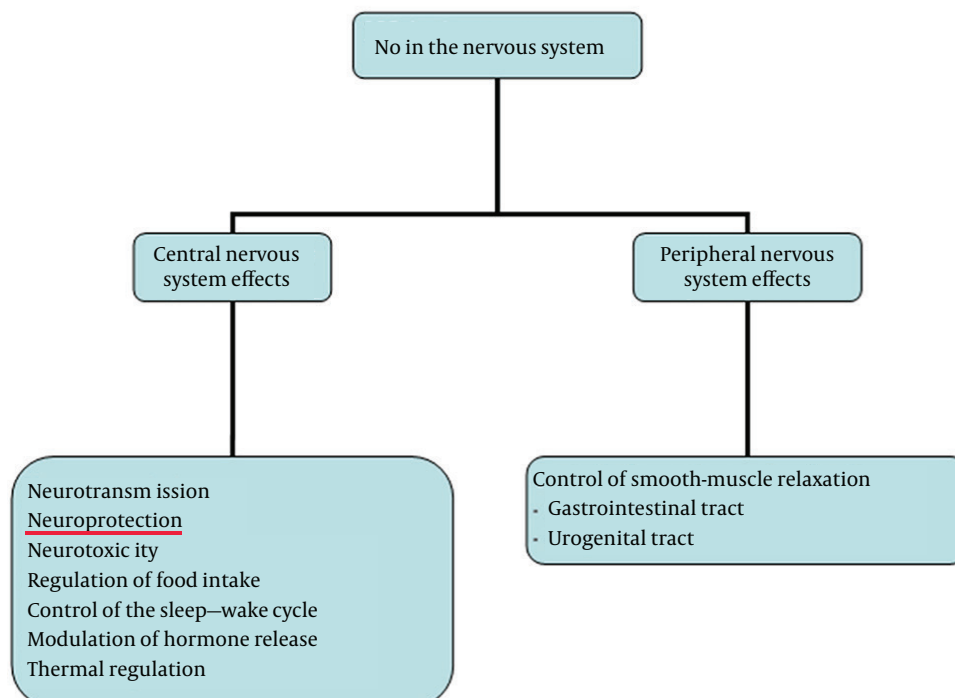

Figure 2. Physiological Role of Nitric Oxide in the Central and Peripheral Nervous Systems (70)
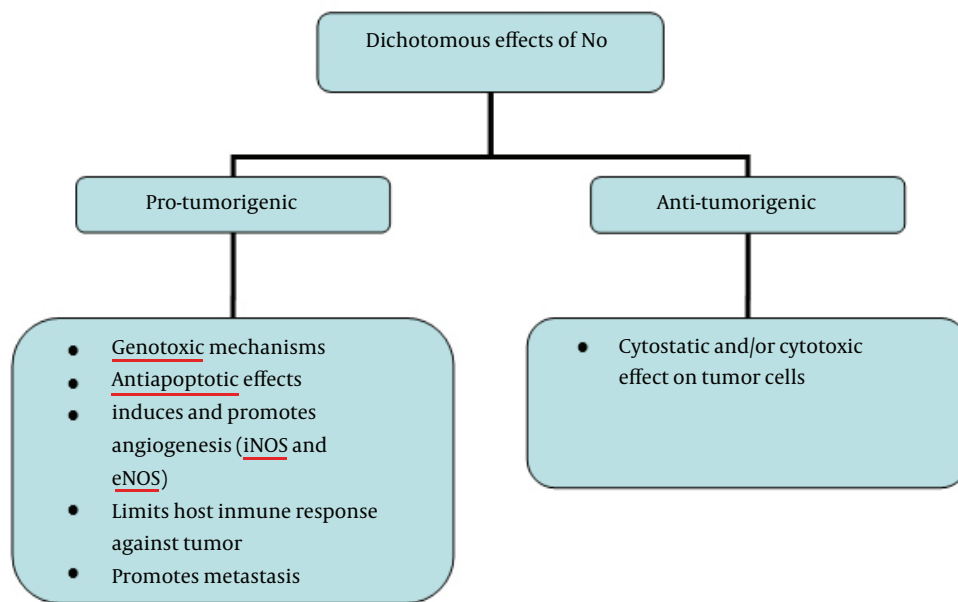
Figure 3. Dichotomous Effects of NO in Cancer

3.7. Nitric Oxide and Metabolic Syndrome

Metabolic syndrome (MetS) constitutes a group of prevalent metabolic risk factors, which raises the risk of diabetes, cardiovascular disease and cancer (91, 92). The components of MetS commonly include obesity, dyslipidemia, hypertension, insulin resistance and elevated fasting glucose. NO level was related to MetS and was loaded with various component of MetS. (17, 19). Some studies both in humans and animals reported an association between MetS and increased NO concentrations (17, 19, 55, 57). High level of NO in MetS subjects occurs as result of eNOS inhibition and iNOS overexpression (17) and may be a compensatory response to oxidative stress and increased insulin levels (17, 55), which could elevate NO release from endothelial cells (55). In contrast, Kowalski et al. showed decreased serum concentration of NO in subjects with MetS (58).

3.8. Nitric Oxide and Hyperglycemia

Endothelial dysfunction in hyperglycemia is a hallmark for the development of atherosclerosis. In type II diabetes mellitus due to insulin resistance and prolonged exposure to high glucose level, elevated eNOS gene and protein expression as well as NO release and stimulatory effect of glucose on NO synthesis may play a role in the pathogenesis of B cell dysfunction in diabetes (4, 59). Excess NO is associated with increased superoxide onion production, which mediates endothelial dysfunction in diabetes. NO, superoxide and peroxynitrite play significant roles in diabetes (4). In addition, NO may have effects on glucose metabolism independent from its vascular ac-

tions (59). High level of NO in men with dysglycemia and hypertension have been found, which indirectly reflects endothelial dysfunction (13, 59). Increased NO has been reported in patients with diabetes (4, 59). Moreover, eNOS and nNOS genes disruption improve insulin resistance in mice; low level eNOS and nNOS may contribute to insulin sensitization (60).

3.9. Nitric Oxide and Obesity

Obesity is strongly associated with cardiovascular atherosclerosis, type II diabetes and insulin resistance. The association between obesity and endothelial dysfunction has been reported (61, 62). In obese subjects, endothelial injury can occur due to decreased bioavailability of NO generated by interaction excessive superoxide anion with NO production by iNOS (62). iNOS is significantly expressed in fat of obese rats and mice; iNOS overexpression mediates obesity-related insulin resistance in skeletal muscle. In addition, iNOS genetic disorder protects obese mice from improving insulin sensitivity in whole body and develops muscle insulin resistance and glucose tolerance (60). However Kraus et al. showed no significant association between elevation in skeletal muscle iNOS and insulin resistance in obese human (63).

3.10. Nitric Oxide and Hypercholesterolemia

Hypercholesterolemia as well as atherosclerosis is associated with an impaired endothelial function, which may be due to impaired production and bioactivity of endothelial NO; interactions between stimulatory Ca^{2+} calmodulin complex and inhibitory protein caveolin re-

ciprocally regulate eNOS activity (64, 65). John et al. found that subjects with hypercholesterolemia taking L-NMMA therapy significantly showed improved endothelium-dependent vasodilation as a result of increased NO bio-availability (66). In addition, some studies reported that decrease in bioactivity of endothelium-derived NO and down-regulation of eNOS are associated with increased levels of LDL by enhancing caveolin-eNOS interaction (64, 67). Pharmacological NOS inhibitors decreased the expression of caveolin in endothelial cells (67).

4. Conclusions

All three NOS isoforms play important roles as dichotomous effects in human biology and diseases. Its effects depend on NO concentration and composition of intra- and extracellular milieu. Although low concentration of NO has beneficial effects, high levels or concurrent presence of ROS can cause toxic effects. Under physiological conditions, NO stimulates guanylate cyclase to generate cGMP, which regulates many cellular activities by affecting intracellular calcium concentrations. In pathological conditions, NO is produced in high levels and converted to peroxynitrite by interacting with superoxide anion. Elevated peroxynitrite has cytotoxic effects and associated with many diseases. There is an important association between serum/plasma NO levels and cardiovascular disease, hypercholesterolemia, heart failure, myocarditis, myocardial ischemia, atherosclerosis, hypertension, diabetes, thyroid disorders, metabolic syndrome and obesity. In addition, NO has a strong trace in neurodegenerative diseases such as Huntington's, Multiple sclerosis, Parkinson's, Amyotrophic lateral sclerosis, Alzheimer's disease and cerebellar degeneration.

Authors' Contributions

Study concept, design and drafting of the manuscript were performed by Dr. Marjan Khazan. Acquisition of data and critical revision of the manuscript for important intellectual content were performed by Dr. Mehdi Hedayati. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

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