

Nitric Oxide- An Inflammatory Biomarker in Oral and Periodontal Diseases

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ABSTRACT

The purpose of our review is to explain the role of nitric oxide (NO) in periodontal tissues in various physiological and inflammatory conditions. NOS can be categorized into three well known isoforms two are constitutive (cNOS) and the third one is inducible (iNOS). When NOS enzymes are cloned it indicated that cNOS showed the presence of both brain constitutive (NOS1) and endothelial constitutive (NOS3); the third is the inducible (NOS2) gene. The constitutive isoforms are available under physiological state. The expression of iNOS is induced in inflammatory conditions, such as periodontitis as well as in gingivitis. Its presence can be well detected in the circumdental tissues as well as in saliva. NO expressed by peroxynitrite, reaction as a byproduct proved its pernicious effects when defined in large numbers in inflammation. The basic role of iNOS production in periodontitis is presumably to express NO and kill the breaching microorganisms. However, when produced in excessive amounts can destroy the host tissue as well. Selective blockage of iNOS might be of therapeutic use in periodontal diseases.

KEYWORDS: Nitrous Oxide, Biomarker, Periodontal Diseases

INTRODUCTION

Nitric oxide (NO) is an intercellular messenger molecule which shows its pervasiveness along with many applications in cardiovascular, neurological and immune functions.¹ Most of the physiological roles which NO mediates are carried via activation of soluble guanylate cyclase which in turn is responsible for its signal transduction. Nitric oxide is synthesized from L-arginine by a family endothelial NO is produced in mammalian cells by a group of isoenzymes which is collectively known to be NO synthases (NOS). All of whose forms are catalyzed by the conversion of L-arginine to L-citrulline in an NADPH dependent pattern, producing NO from the terminal N-guanidino group of L-arginine² which is derived as a relaxer of vascular smooth muscle, and of enzymes called nitric oxide synthases.

Nitric oxide (NO) is a short living product of nitrogen metabolism, produced by many cells in the organism with much important physiological function.³ NO is produced most prominently in endothelial and neural cells whereas Macrophages and other inflammatory cells can induce its synthesis and release. The most prominent carriers of NO synthesis are bacterial products.⁴ The known biological functions of NOS can be divided into two categories. One which acts as a relaxer of vascular smooth muscle of endothelial origin, also prevents adhesion and aggregations of platelets and is a neuronal messenger. Secondly, ability of the NO to kill various bacteria, viruses, protozoa and tumor cells is carried by activating macrophage which is a known cytotoxic molecule. In addition, it is well established that nitric oxide secreted

by macrophages has damaging effects for cellular proteins, DNA, and lipids leading to periodontitis.⁵ NOS exists as three distinct isoforms, namely, endothelial NOS (eNOS), neural NOS (bNOS), and inducible NOS (iNOS).⁶ eNOS and bNOS are collectively known as constitutive (cNOS) and release small amounts of NO for short periods followed by the stimulation of any of the receptor. When compared to cNOS, iNOS is expressed in response to proinflammatory stimuli and produces large amounts of NO for a sustained duration. NO, when produced in greater concentrations proves to be crucial in nonspecific host defense, along with its various cytotoxic activities against fungal, bacterial, and protozoal organisms as well as tumor cells. Recent researches on nitric oxide indicated no correlation in its toxicity independently, and formation of peroxynitrite might be one of the important factors for it being cytotoxic.⁷

A chronic inflammatory disease of the periodontal tissue is known as periodontitis one of the most frequently occurring diseases of human tissues which is of bacterial origin. Periodontitis is frequently initiated by an overgrowth of some gram-negative bacteria in the dental pocket which often leads to inflammation, destruction of the periodontal tissue and tooth loss. Various clinical and epidemiological studies suggest that periodontitis is associated with an increased risk of cardiovascular diseases (CVDs).⁸ Periodontitis generally leads to an increase in production of iNOS and NO production in gingival tissue (Rausch-Fan & Matejka 2001), whereas locally produced NO is cytotoxic against periodontal

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pathogens and the tooth surrounding tissue (Kendall et al. 2001). Various studies related with the levels of NO metabolites in the saliva of periodontitis patient, results presented both an increase as well as decrease in the level of salivary metabolites of NO in periodontitis patients. (Aurer et al. 2001, Reher et al. 2007, Ozer et al. 2011, Parwani et al. 2012). A positive correlation is found in between Periodontitis and an increased risk of CVDs, which is usually accompanied by impaired NO production by eNOS (Vallance & Collier 1994, Wennmalm 1994). The only study shown to have a positive increase of serum nitrite levels in periodontitis (Menaka et al. 2009). However, when nitrite measured alone in serum shows no deflection in the levels of NO production, as nitrite is one of the few molecule which gets oxidized to nitrate (Moshage et al. 1995)

CLASSIFICATION

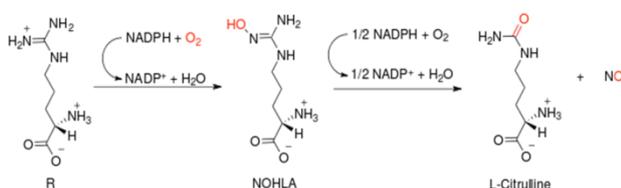
Different members of the NOS family are encoded by separate genes.⁹ NOS can be categorized into three well known isoforms two are constitutive (cNOS) and the third one is inducible (iNOS).¹⁰ when NOS enzymes are cloned it indicated that cNOS showed the presence of both brain constitutive (NOS1) and endothelial constitutive (NOS3); the third is the inducible (NOS2) gene.¹¹ Recently a study, including notorious pathogens like *Bacillus anthracis* and *Staphylococcus aureus* demonstrated the presence of NOS activity.¹² (Table 1)

Name	Gene	Location	Functions
Neuronal NOS (nNOS or NOS1) NOS1	(Chromosome 12)	nervous tissue skeletal muscle	type II cell communication
Inducible NOS (iNOS or NOS2) NOS2	(Chromosome 17)	Immune system Cardiovascular system	defense against pathogens
Endothelial NOS (eNOS or NOS3 or cNOS) NOS3	(Chromosome 7)	endothelium	Vasodilation
Bacterial NOS (bNOS)	Multiple	various Gram positive bacteria	defense against various oxidative stress antibiotics, immune attack

Table 1: Classification for different forms of NO synthase

CHEMICAL REACTIONS OF NOS

Nitric oxide synthases generates NO by catalysing a five-electron oxidation of a guanidino nitrogen of L-arginine (L-Arg). Oxidation of L-Arg to L-citrulline is brought about through two successive monooxygenation reactions producing *N*^ω-hydroxy-L-arginine (NOHLA) as an intermediate by product 2 mol of O₂, and 1.5 mol of NADPH are consumed per mole of NO formed.¹³



FUNCTIONS OF NOS

Vasodilation: Nitric oxide (NO) is of critical important for its role in vasodilation of blood vessels which is induced by several factors, and once when synthesized by eNOS it results in phosphorylation of several proteins which in turn leads to relaxation of the smooth muscle.¹⁴ An essential element for the regulation of blood flow, blood pressure and also for the control of homeostatis nitric oxide and vasodialation has shown a positive correlation.¹⁵

Induction: Endothelial nitric oxide synthase (eNOS) produced by the stimulation of NO via the activation of various factors like Platelet-derived factors, shear stress, acetylcholine, and cytokines .eNOS synthesizes NO from the terminal guanidinenitrogen of L-arginine and oxygen and yields citrulline as a byproduct. NO production by eNOS is dependent on calcium calmodulin and other cofactors.

Phosphorylation: NO, interacts with soluble guanylate cyclase which is present in the smooth muscles cells of blood vessel. A second messenger cyclic GMP (3',5' guanosine monophosphate) is generated from guanosine triphosphate (GTP) which is stimulated via nitric oxide, which in turn activates cyclic nucleotide dependent protein kinase G (PKG or cGKI). PKG phosphorylates various proteins which regulate calcium concentrations and sensitization, potassium channels hyperpolarizes cells, and also cause actin filament and myosin structural alterations which leads to smooth muscle relaxation.¹⁶

Penile erection: Vasodilatory effect of NO also plays a critical role in development and maintenance of penile erection. The corpus cavernosum which is supplied by various blood vessels when dilated leads to more blood flow and, hence, penile erection .An enzyme phosphodiesterase is inhibited in the use of sildenafil (Viagra) that eventually lowers the cGMP concentration by reverting back GMP.

Immune system: Macrophages, defense cells of the immune system, produces NO as an protective barrier for destruction of the invading bacteria's. The nitric oxide synthase is inducible NOS form is the isoform utilized in this case. Various circumstances leads to it reversion like sepsis which leads to an excess in production of nitric oxide by macrophages, causing dilation of blood vessels which is, probably one of the rationale for low blood pressure presentation in cases of sepsis. The cytotoxic levels of nitric oxide is seen in with an increased expression of inducible form of NO.¹⁷

Neurotransmission: Nitric oxide is a small uncharged and fat soluble molecule which acts as a neurotransmitter. Unlike other neurotransmitters it not only does transmit information from a presynaptic to postsynaptic neuron but can diffuse widely and distribute it hence it can actively play its role on several nearby neurons even on those not connected by synpse. It also plays a vital role in redox signaling. Simultaneously the short life NO proves

that any of such actions will be confined to a specific area, without the inevitability for its enzymatic breakdown or cellular reuptake. NO is also highly reactive with other free radicals, lipids, and proteins. NO is also involved in learning and memory through the maintenance of long-term potentiation (LTP) via NOcGMP cascade. Nitric oxide which is an important nonadrenergic, noncholinergic (NANC) neurotransmitter for few zones of the gastrointestinal tract, which leads to relaxations of GIT smooth muscle and in the stomach, it increases the uptake of the fundus to store food and fluids.¹⁸

Other functions: An important source of dietary nitrate in mammalian food includes Green, leafy vegetables and some root vegetables, (such as beetroot)¹⁹. Nitrate is found in higher concentration in saliva and is reduced to nitrite onto the surface of tongue as a biofilm containing various facultative anaerobic bacteria after its absorption into the blood stream.²⁰ The aim behind this mechanism is to create a biofilm of NO which acts both sterilization of swallowed food, and to maintain mucosal blood flow of the gastric region and also prevent food poisoning.²¹ A similar cycle is seen in the layers of skin where nitrate is produced in sweat reduces to nitrite and eventually to NO by the commensal microorganisms present on the skin. Similarly when skin is exposed to sun, nitrite anions get photolyzed to the free nitric oxide radicals via the ultra-violet radiations from the sunlight.²² The therapeutic purposes²³ are vastly affected through this mechanism which many elicit various changes in the systemic blood circulations. NO also shows its effect onto the cardiac muscles which leads to reduction in regulatory and contractility of cardiac muscles. Various studies conducted for correlation of coronary artery disease (CAD) showed its relative action in production of defects in production or action of NO.²⁴ Reduced levels of exhaled NO have been associated with exposure to traffic related air pollution.²⁵ The bacterium *Deinococcus radiodurans* have shown to hold on stresses and a higher level of radioactivity. In 2009 it was reported that NO plays a key role in the recovery of any bacteria after radiation exposure, whenever DNA damage as being repaired a gas is required for its proliferation and division. After the exposure of skin to UV radiations a gene was found to increase the production of NO and in the absence of this gene the bacteria still repaired its DNA damage but weren't able to multiply further.²⁶

Diabetic patients: People with higher blood glucose level usually have higher levels of NO when compared with patients with normal or slightly elevated blood glucose level. In absence of this nitric oxide there is vascular damage and inflammation. When the vascular damage is extensive it can lead to decrease in blood flow to extremities which increases the risk of the diabetic patient to be more likely to develop neuropathy, nonhealing ulcers and increases the risk for limb amputation as well.²⁷

Pharmaceutical analogs: The pharmaceutical analogs used in the treatment of various heart diseases including Nitroglycerin, amyl nitrite, "poppers" (isobutyl nitrite or similar), and other nitrite derivatives. These compounds are actively converted to nitric oxide (by a mechanism that is still not completely understood), which in return dilates the coronary artery, because of which there is an increasing in its blood supply. These analogs are basically venodilators one which dilates the peripheral veins and thus directly leads to an reduction in venous return along with the preload to the heart. Thus there is an reduction in oxygen requirement of the myocardium and subsequently reduction in the angina pain which is felt during myocardial ischemia.²⁸

Method Of Detection: There are various methods to detect salivary nitric oxide in samples²⁹. They are

- Griess reaction
- Electrochemical reaction
- Chemiluminescent Detection of NO

ROLE OF NITRIC OXIDE SYNTHASE IN ORAL DISEASES AND PERIODONTITIS

The basic reaction of the reactive oxygen species in periodontal disease is the respiratory burst mediated by PMN. These leucocytes are cells predominantly located in the furrow of gingival epithelium also in the adjoining connective tissue. Whenever there is an increase in the activity of PMN because on any local inflammatory condition like dental plaque there is a migration of the neutrophils and gingival fluid, which activates the leucocytes by the outburst of reactive oxygen species (HOCl, superoxide radical anion, etc.) in the soft tissue. There are various morphological as well as functional changes occurring in the periodontal tissues and their blood vessels. Which is mediated by the lipid peroxidation process, where amino acids (part of the extracellular matrix) and the glucosamine-glycane chains depolymerize because of ROS action, which ultimately leads to collagen destruction and the bone tissue reabsorption as its terminal step.³⁰

During ROS process many pro-inflammatory cells, fibroblasts, endothelial vascular cells and osteoclasts are produced. The production of superoxide which is then converted to hydrogen peroxide, hydroxyl radical and single oxygen molecule. A high amount of concentrated nitric oxide is produced by and aggravated response of inducible nitric oxide synthase (iNOS) as a revert mechanism to inflammation.

This elevated response of iNOS in periodontal tissues proves nitric oxide participation in various disease progression. Tissue destruction is clearly evident when high amounts of NO is produced which acts as cytotoxic for both the microbial pathogens and tissues. This rightly

proves NO activity to play a critical role against various infectious diseases which does include periodontitis. Arginine is a semi-essential amino acid utilised as a substrate by both arginase and NOS its increases activity may cause reduction in production of NO leading to increased activity of the bacteria.³¹

Bacterial plaque are responsible for the production of toxins, enzymes and metabolites which play a vital role in the initial process of inflammation. The lipopolysaccharides of the gram negative bacteria or inflammatory markers produced by the neutrophils enhance the the production of iNOS through the cells.³¹

Leyla Ozer, Serenay Elgun, Burcu Ozdemir, Beste Pervane, and Nurdan Ozmeric³² conducted a study to evaluate arginine-NO-polyamine pathway alteration in human saliva and gingival tissue biopsy of patients with mild to moderate gingivitis or periodontitis compared to healthy controls groups and measured the response to periodontal treatment. In which they obtained an increased in salivary NO levels significantly in the periodontitis group and decreased in the gingivitis group. Hence they conducted a greater affinity in the gingival tissues towards periodontal pathogens as compared to those pathogens in saliva. Also NO arginase was measured in increased amounts where an established lesion of periodontitis was found to those compared to an early phase.

Lohinai et al and Lappin et al³³ in 2000 mentioned an increased in amounts of iNOS expressed in mast cells, lymphocytes and microphages in experimentally induced periodontitis in mice as well as in mature mast cells and endothelial cells where in human study periodontitis suggested the production and participation of NO in periodontal disease.

Parwani SR, Chitnis PJ, Parwani RN³⁴ conducted a prospective study calculating the salivary NO levels in periodontitis and gingivitis. A total of 90 subjects were included in the study. Non stimulated salivary samples were collected from each subject, and NO levels were assayed by Griess reaction. They found that the NO levels were fluctuating significantly in gingivitis and periodontitis subjects when compared with healthy. NO showed reduction in its level as inflammation subside in each of the study groups healing period. In patients having periodontitis salivary NO levels showed an affirmative inter-connection in probing pocket depths and salivary NO. hence it proved that Nitric Oxide could be used as a good indicator in evaluating the process of inflammation in periodontium.

K. B. Meneka, Amitha R, Biju Thomas, N Sucheta K³⁵ also conducted study to examine the level of NO in blood serum and chronic periodontitis and to compare these levels with the severity of periodontal destruction. A total number of 60 subjects were included in this study when NO levels were assayed with griess reaction and measuring the accumulation of stable oxidative metabolites nitrite. They found patients with periodontitis had increased levels of nitrite in serum when compared to

group of healthy subjects. Thus, it was concluded that increased presence of NO provided knowledge about the disease progression.

CONCLUSION

Low concentrations of basally produced NO provide with a helpin hand in maintance of normal homeostatis host tissues and are a protective barrier of circumdental tissues under physiological conditions. perhaps, NO may prove to be mischievous when produced in an increasing amounts during inflammatory, which may lead to the destruction of host tissues along with the invading bacterias. Selective blockage of iNOS and maintaines of cNOS production might be of therapeutic use in periodontal diseases.

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