Review Article

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Nitric Oxides in Periodontics

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ABSTRACT

Background: Nitric oxide (NO) is a free radical, that is, an uncharged molecule with an unpaired electron. NO is a molecule, involved in vascular regulation, homeostasis, bone formation and resorption, neuro-transmission, and immune function. It also has antimicrobial activity and is considered to be an important molecule against infectious diseases, such as periodontitis. NO works as an effector of host-induced tissue damage mediated by the production of proinflammatory cytokines, prostaglandins and by the production of reactive nitrogen species. It might play a role in the pathogenesis of periodontitis and subsequent bone loss, either directly or indirectly by modulating the production of other pro-inflammatory cytokines. Although little has been published on its role in oral diseases, it certainly has important actions in periodontal diseases. Hence, this review will throw a light on the role of nitric oxide in periodontal disease based on recent evidences.

Keywords: Nitric oxide, Free radical, Periodontitis

INTRODUCTION:

Nitric oxide (NO) is a free radical, that is, an uncharged molecule with an unpaired electron. It's a Ubiquitous intercellular messenger molecule with important cardiovascular, neurological and immune functions.

NO was thought to be one of the oldest molecules on earth, being formed in the primitive atmosphere of the cooling planet[1]. A Belgian scientist, Jan Baptista van Helmont was credited as the first to synthesize NO in the laboratory in about 1610. In 1772, Joseph Priestley gave it the name 'nitrous air' and observed that it did not support plant life, but reduced putrefaction in meat exposed to it. It was not until 1987 that NO was discovered to be the chemical responsible for the actions of endothelial derived relaxing factor (EDRF).[2,3] Following this finding, NO research expanded exponentially, and it is regarded by many in the scientific community as one of the greatest discoveries of the 20th century. In recognition of this, two Nobel prizes have been awarded to researchers in the NO field, and it was named as molecule of the year by the Journal Science in 1992.

NITRIC OXIDE BIOSYNTHESIS

NO is one of the few gaseous signalling molecules known and is additionally exceptional due to the fact that it is a radical gas. NO is biosynthesized endogenously from L-arginine, oxygen by various nitric oxide synthase (NOS) enzymes. The amino acid L-arginine is the substrate for the NOS enzymes, generating NO and the by-product L-citrulline[4].

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There are three forms:

- Type 1 nitric oxide synthase – brain enzyme (bNOS);
- Type 2 nitric oxide synthase – inducible enzyme (iNOS), found in macrophages;
- Type 3 nitric oxide synthase – endothelial cell enzyme (eNOS).

The constitutive isoforms were originally located in eNOS and neurones (ncNOS), the activity of which is dependent on elevated intracellular calcium levels modulated through calmodulin[5]. The inducible isoform (iNOS) is a calcium-independent, cytosolic enzyme produced largely by cytokine activated macrophages and many other cells[6].

Since there is complex and overlapping expression of NOS isoforms, not necessarily specific to cell type, a numerical classification of NOS has been proposed, which does not specify cell of origin and takes into account the differing NOS genes.

Table 1: Origin and Functions of NOS-II

NOS-II upregulation	NOS-II downregulation	Cell types include
IL-1, IFN-g, TNF-a, granulocyte/macrophage TGF-b, stimulating factor, LPS, synergistic response-LPS/IFN-g	Glucocorticoids, IL-10, IL-4, NO	Macrophages, Hepatocytes, Chondrocytes, Endothelium Fibroblasts. Osteoblasts, Osteoclasts, Keratinocytes

HK Kendall et al. Nitric oxide and tissue destruction. Oral Diseases (2001) 7, 2–10

ROLE OF NITRIC OXIDE

1. The first role discovered was in the regulation of the blood vessel relaxation.
2. It also helps in dilating blood vessels.
3. It reduces platelet stickiness.
4. It reduces monocyte stickiness (prevents formation of plaque).
5. It reduces multiplication of smooth muscle cells of the artery wall.
6. It reduces the release of superoxide radicals.
7. It reduces oxidation of LDL cholesterol which is a major component of atheromatous plaque.
8. NO is also generally involved in bone formation and resorption, neurotransmission and immune function.

NITRIC OXIDE AND ORAL DISEASES

Although there have been few publications concerning this anatomical area, interest in NO is expanding rapidly.

1. Odontogenic cysts and periapical infection:

There has been only one paper to date that has investigated NOS expression in odontogenic cysts. This study investigated the expression of NOS2 in radicular cysts[7].

2. Periapical pathology:

NO has also been implicated in the pathogenesis of apical infection[8]. Again, inflammation-induced NOS2 accounts for NO production, and it has been postulated that NOS inhibitor drugs introduced into the root canals of teeth with periapical infections may lead to resolution of this condition[7].

3. Oral Lichen Planus (OLP):

OLP itself is a complex disorder producing many cytokines. It was postulated that the balance between NOS2 activation and inhibitory cytokines in OLP favoured NOS2 inhibition over activation, but clearly further research is required in this field[9].

4. Salivary Gland Diseases:

Bentz et al. assessed NOS3 expression in a variety of benign and malignant salivary tumours and found increased NOS3 expression in all 48 tumours studied relative to normal salivary tissue where little NOS3 was found outside blood vessel endothelium[10]. In a recent study NOS2 was expressed both in pleomorphic adenoma and normal salivary ducts, and postulated that its

expression in this tumour was due to its origin from myoepithelial cells[11]. The significance of these findings is as yet unknown as are the effects of NO in saliva.

5. In Carcinogenesis:

Since high concentrations of NO cause genetic mutations, it could be postulated that long-term NO exposure (from NOS2 activity during chronic inflammation) could have an active role in carcinogenesis. In many of the studies conducted on oral cancer, tumour cells were the predominant source of NO2 expression[12,13].

NITRIC OXIDE AND PERIOODNTITIS

It is considered that the primary source of Reactive oxygen species (ROS) in periodontal disease is the "respiratory burst" of activated PMN. These are the predominant leucocytes in the gingival furrow epithelium and the adjacent connective tissue. Under certain conditions the local factors (dental plaque) induce the migration of the gingival neutrophils and the gingival fluid. This infact induces a rupture of periodontal soft tissues by ROS (HOCl, superoxide radical anion, etc.), caused by PMN activation. The lipid peroxidation process, the amino acid functional groups oxidation (part of the extracellular matrix) and the glucosamine-glycane chains depolymerization mediated by ROS action, represent the starting mechanism in the development of morphofunctional change in the periodontal tissues and their blood vessels. The collagen destruction and the bone tissue reabsorption are the final steps.[14]

Many inflammatory cells, fibroblasts, endothelial vascular cells and osteoclasts also produce ROS. The superoxide generated is converted to hydrogen peroxide, hydroxyl radical and singlet oxygen. The expression of the inducible nitric oxide synthase (iNOS) in response to an inflammatory stimulus produces a high concentration of nitric oxide.

The increase in iNOS activity in periodontal tissues suggest the production and participation of NO in the disease process. When NO is locally produced in high amounts by iNOS, it can act as a cytotoxic molecule against microbial pathogens and surrounding cells possibly leading to tissue destruction. Thus, NO has antimicrobial activity and is considered to be an important molecule against infectious diseases, such as periodontitis. Because arginine is used as a substrate by both arginase and NOS, an increase in arginase activity may lead to a reduction in the production of NO leading to an increased susceptibility to bacterial infections[15].

The toxins, enzymes and metabolites of the bacterial plaque play a key role in the initiation of the inflammatory process. The endotoxins of the gram negative bacteria or proinflammatory cytokines produced by the inflammatory cells trigger immigrant cell population for the expression of iNOS.

Leyla Ozer, Serenay Elgun, Burcu Ozdemir, Beste Pervane, and Nurdan Ozmeric[16] did a study which examined the arginine-NO-polyamine pathway alteration in saliva and gingival biopsy samples of patients with gingivitis or periodontitis compared to healthy controls and evaluated the response to periodontal treatment. Salivary NO levels significantly increased in the periodontitis group and decreased in the gingivitis group. They concluded that gingival tissue seems to be more informative about periodontal pathogenesis than saliva. And at an early phase of periodontal inflammation, NO arginase was measured higher than at an established lesion of periodontitis.

Lohinai et al and Lappin et al[17] in 2000 stated that high amounts of iNOS expression in macrophages, lymphocytes and PMN in experimentally induced periodontitis in mice as well as in macrophages and endothelial cells in human periodontitis suggest the production and participation of NO in periodontal disease.

Parwani SR, Chitnis PJ, Parwani RN[18] conducted a case control study estimating salivary NO levels in inflammatory periodontal diseases. A total of 90 subjects were included in the study. Saliva samples were collected from each subject, and NO levels were assayed by Griess reaction. The results showed that the NO levels were increased significantly in gingivitis and periodontitis subjects as compared with controls. There was a statistically significant decrease in the NO levels in each study group after the healing period (corresponding to the reduced clinical signs of inflammation). A positive correlation was found between probing

pocket depths with salivary NO levels in periodontitis group. It concluded that salivary NO levels can be utilized as a good indicator of the inflammatory status of the periodontium.

K. B. Meneka, Amitha R, Biju Thomas, N Sucheta K[19] did a study to assess the level of NO in serum and chronic periodontitis and to correlate these levels with the severity of periodontal disease. 60 patients participated in this study and NO levels were assayed by measuring the accumulation of stable oxidative metabolites nitrite with Griess reaction. The results showed subjects with periodontitis had higher levels of nitrite in serum compared to healthy subjects. Thus increase in the NO production enabled to understand its role in disease progression

CONCLUSION

There has been an exponential rise of interest in NO since its discovery as a biological messenger in 1987. Although relatively little has been published on its role in periodontal diseases, it almost certainly has important and damaging actions. Despite over 50,000 published papers to date on NO, this tiny molecule still holds many mysteries.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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