



Nitric Oxide: An Overview

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ABSTRACT

The small molecule nitric oxide (NO) has a vast number of actions, many of which are poorly understood. Although NO is produced by three distinct isoforms of the enzyme nitric oxide synthase (NOS), most research is directed toward the form, iNOS which is seen following induction. Nitric oxide has been extensively researched in relation to cancer, where it has a multifaceted role. It has also been investigated in relation to oral lesions and tumors like ameloblastoma, salivary gland tumors, periapical lesions, Sjogren's syndrome, etc. This review looks into all these facets of NO and its potential role as a diagnostic and therapeutic modality.

Keywords: Cancer, Angiogenesis, Dysplasia.

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INTRODUCTION

Nitric oxide (NO) is a small gaseous molecule, which was considered to be only an air pollutant till as recently as 1987. It was Palmer et al who first reported that the endothelium derived relaxing factor was nothing but NO, this discovery led to an exponential amount of research activities related to NO making it the molecule of the year in 1992.¹ Nitric oxide has undergone a drastic change of image in recent times, i.e. from being considered to be only an air pollutant, to its present day status of a physiological mediator of health and disease. At basal levels, it is an essential, short lived intercellular messenger; however, at elevated levels, it causes disruption of intracellular signaling and metabolic function, resulting in cell death.²

With more than 30,000 papers already published on NO, it is currently one of the fastest growing branches in biomedicine, interestingly only a few papers related to head and neck pathology are available in the literature. This review aims to give a brief insight into the basic pathophysiology of this fascinating molecule, and also focusing on a few areas of interest to oral pathologists.

PATHOPHYSIOLOGY

Nitric oxide is involved in a wide repertoire of biologic actions, its physiologic roles include vascular relaxation, inhibition of platelet aggregation, macrophage mediated cytotoxicity, neurotransmission and many other actions most of which are poorly understood. Nitric oxide is readily able to pass through cell membranes and influence enzymes and proteins in both the cytosol and the nucleus. Nitric oxide is synthesized from the amino acid L-arginine, by the action of enzyme nitric oxide synthase (NOS) in presence of cofactors like NADPH and FAD. At present, three forms of this enzyme have been reported each one being a product of a different gene (Table 1).³

Nitric oxide synthase activity has been demonstrated in several bacterial species, including notorious pathogens *Bacillus anthracis* and *Staphylococcus aureus*.⁴

- Type I NOS is also known as nNOS because it was first isolated from neural tissues, the nerves which express NO are referred as NANC nerves (i.e. nonadrenergic, noncholinergic nerves) or nitrergic nerves, these are found innervating cerebral and penile arteries.
- Type II NOS is also referred as iNOS, 'i' stands for inducible, immunological or independent.
- Type III NOS is also known as the eNOS, 'e' stands for endothelial as this isoform was first isolated from endothelial cells. Type I and III enzymes are also called as constitutive enzymes as they are expressed continuously in the cells. They are bound to the cell membrane and are dependent on tissue calcium level for their activity, in contrast to these enzymes the third enzyme, i.e. type II NOS is a cytosolic enzyme which is induced in response to release of cytokines, such as IL-1, INF-gamma, TNF-alpha and also by lipopolysaccharide, as iNOS activation results in prolonged production of NO and elevated level of iNOS is associated with many lesions. Most research is oriented toward this isoenzyme. Nitric oxide has got a very short half life and hence its presence in

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Table 1: Types of nitric oxide synthase

Name	Gene(s)	Location	Function
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	Immune defense against pathogens
Endothelial NOS (eNOS or NOS3 or cNOS)	NOS3 (chromosome 7)	Endothelium	Vasodilation

tissues is very difficult to detect, an easier method is to assay the activity of NOS in tissue sections using the principles of immunohistochemistry.

EMERGING ROLE OF NITRIC OXIDE IN CANCER

Tumors induce vascularization, which is essential to sustain their growth by providing supply of oxygen and nutrients and to maintain a suitable pH. Inducible nitric oxide synthase (iNOS) has been shown to be localized to the endothelium of tumor microvasculature. The NO produced by tumor cells appears to maintain their blood flow and prevent platelet aggregation.

In tumor biology, NO plays a variety of roles which are at times contradictory; on one hand, NO is involved in different etiological mechanisms as well as promoting tumor growth by angiogenesis; on the other hand, NO derived from leukocytes plays a major role in their tumoricidal activity. It depends on its timing, location, and concentration. It has been reported that intensive iNOS activation and NO overproduction may induce apoptosis and suppress tumor growth, but there is now a growing body of evidence to suggest that the amount of NO produced by human tumors is atleast one to two times less than that is necessary to be cytotoxic to tumor cells. Nitric oxide is also reported to inhibit metalloproteinases thus reducing the ability of tumors to metastasize.⁵

As it is the case of tumor progression and metastasis, NO has a multifaceted role in anticancer therapy as well. The tumoricidal and normal tissue toxicity of radiation therapy and chemotherapy involves rapid interaction of NO radical with the superoxide radical to form an intermediate product peroxy nitrite anion (OONO⁻), this anion further decomposes to nitrogen dioxide radical (NO₂) and hydroxyl radical (OH⁻), studies conducted by substituting NO gas for oxygen have shown that NO is as effective as oxygen, with an additional advantage being that when NO is used, peroxy nitrite anion is produced which diffuses further than hydroxyl radical (OH⁻) and decomposes in an acidic environment, thus allows (OH⁻) to react with cell constituents away from the oxygenated part of the tumor.⁶

In cancer therapy with use of bioreductive chemotherapeutic agents, it is beneficial to have low levels of

NO, as this makes the cells hypoxic and hence more susceptible to the effect of these chemotherapeutic drugs. Thus, maximum therapeutic gain can be obtained by increasing the levels of NO by using NO donors, such as nitroglycerine or by decreasing the NO levels by using arginine inhibitors, depending on the mode of treatment. Most cytotoxic therapies mediate their activity primarily by inducing apoptosis, many patients who initially responded to conventional therapies experience relapse and recurrences. This resistance may be explained by development of resistance to apoptosis by activation of an antiapoptotic pathway, such as nuclear factor-kappa B (NF-κB).⁷ Nitric oxide has been shown to inactivate NF-κB by inhibition of P50; hence, NO can sensitize the tumor cells to chemotherapy, immunotherapy and radiotherapy.⁸

ROLE OF NITRIC OXIDE IN ORAL LESIONS

Nitric oxide levels have been reported to be increased in a wide array of oral lesions. Oral premalignant and malignant lesions, such as oral submucous fibrosis, verrucous hyperplasia, verrucous carcinoma, oral lichen planus, squamous cell carcinoma, etc. have shown an increased expression of iNOS;^{9,10} moreover, the degree of expression of iNOS correlates with the severity of epithelial dysplasia.¹¹ Studies have also reported that psychological stress causes NO release in correlation with increase of neural NOS activity, this may also lead to increase in severity of oral lesions. Increased levels of NO are also reported in odontogenic tumors, such as malignant ameloblastoma and salivary gland tumor's and diseases, such as pleomorphic adenoma, Warthin's tumor, Sjogren's syndrome, etc.^{12,13}

In periapical inflammatory lesions increased iNOS expression was detected in the epithelial cells and it is thought that this might play a role in activation and proliferation of lining epithelium leading to progression of periapical lesions.¹⁴

In a comparative study among smokers and non-smokers, it was found that the salivary NO level in smokers was elevated and it was proposed that NO plays a role in pathogenesis of lesions associated with smoking.¹⁵ An increased level of NO is seen in all these lesions compared with a lack of expression in normal tissues suggests that an NO dependent mechanism may be involved in

the pathogenesis of these lesions. As the degree of expression of iNOS correlates with the increase in severity of dysplasia, the use of iNOS immunohistochemistry may, therefore, assist us pathologists as an additional tool in helping to grade the severity of dysplasia.

An interesting area of research concerns the relationship between iNOS and p53. It is found that wild type p53 downregulates the expression of iNOS, a negative feedback loop exists between iNOS and p53, p53 mediates transpression of iNOS gene thus reducing the NO mediated pathways of angiogenesis and tumor progression. Mutation of p53 gene gives the tumors a dual advantage of failure of p53 mediated apoptosis as well as increased levels of NO, which may in turn help in tumor progression.¹⁶ The inhibition of iNOS may provide a target for future therapy. Very few studies have studied the role of NO in oral precancer. The exact role of NO and the concentration in which it is present in oral precancer is being studied intensively. Nitric oxide could stimulate tumor growth and metastasis by promoting the migratory, invasive and angiogenic abilities of tumor cells, which may also be triggered by the activation of cyclooxygenase-2 (COX-2).¹⁷ Recently, much attention has been paid to the role of COX-2 in carcinogenesis. It is also extensively expressed in oral cancer and oral premalignant lesions and seems to be enhanced specifically in high-risk oral lesions.¹⁸ A significantly higher expression level of iNOS was found in the human OSCC.¹⁹ As a result, iNOS generating NO, and might be able to play an important role in oral cancer progression studies are required to know the exact role of NO in oral precancer which will be helpful in preventing the malignant transformation.

CONCLUSION

Nitric oxide has complex and contradictory actions on most of the tissues and hence has been rightly termed by a few authors as a 'double-edged sword' with both beneficial and deleterious effects, depending on the amount and conditions under which it is produced. Current research into the biologic role of NO is revealing that it is a Panacea as well as a Pandora's box and despite an ever-growing number of studies on NO, this small molecule still holds many mysteries, unraveling which would be an very interesting challenge.

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