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Derleme/Review

ROLE OF NITRIC OXIDE IN INFLAMMATORY PERIODONTAL DISEASES: A REVIEW

NİTRİK OKSİDİN İNFLAMATUAR PERIODONTAL HASTALIKLARDA ROLÜ : DERLEME

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ABSTRACT

Nitric oxide (NO) is a unique molecule for biological organisms, and has also very important effects on various pathologic and physiologic mechanisms. Many cell types can generate NO during different pathological and physiologic conditions. Recently, many authors have been reported the connections between NO and inflammatory diseases. Periodontal pathologies are characterized with specific periodontal inflammation and host tissues damage around the teeth, and additional factors such as force application on teeth. However, the production of NO and the role of NO in the development of periodontal pathologies are unclear. On the other hand, many evidences from previous studies indicates a close relationship between NO and periodontal pathologies. In this review, we want to discuss; overproduction of NO during inflammation and mechanical stress of force application on teeth, which directly cause progression of periodontal pathologies, and the possible effects of NO overproduction in periodontal diseases.

Keywords: Nitric oxide, periodontal pathologies, inflammation, force application on tooth.

ÖZET

Nitrik oksit (NO) biyolojik organizmalar için benzersiz bir moleküldür ve ayrıca NO'in birçok patolojik ve fizyolojik mekanizma üzerine önemli etkileri bulunmaktadır. Birçok hücre tipleri farklı patolojik ve fizyolojik koşullarda NO oluşturabilir. Son zamanlarda birçok yazar NO ile inflamatuar hastalıklar arasında ilişki olduğunu belirtmiştir. Periodontal patolojiler özellikle periodontal inflamasyon ile dişlerin etrafındaki ana dokularda hasar ve dişler üzerine uygulanan kuvvetler gibi ilave faktörlerle karakterizedir. Ancak periodontal patolojilerin gelişiminde NO üretiminin ve NO'in rolü açık değildir. Diğer taraftan yapılan çalışmalarda NO ve periodontal patolojiler arasında sıkı bir ilişki olduğu belirtilmiştir. Bu derlemede inflamasyon ve dişler üzerine kuvvet uygulamasında oluşan mekanik stres süresince NO'in aşırı üretimini tartışmayı amaçladık. Bu durumlar direk olarak periodontal patolojilerin ilerlemesinde ve böylece periodontal hastalıklarda NO'in aşırı üretiminde etkili olabilmektedir.

Anahtar Kelimeler: Nitrik oksit, periodontal patolojiler, inflamasyon, dişler üzerine kuvvet uygulaması

INTRODUCTION

NO is a short-lived free radical molecule that has important roles in many physiological and pathological processes. This highly reactive and very simple molecule is produced in variety mammalians cells including endothelium,[1] neuronal cells,[2] smooth muscle cells,[3] macrophages,[4] neutrophils,[5] fibroblasts,[6] hepatocytes,[7] chondrocytes[8] and synoviocytes[9] by oxidation of arginine, an amino acid, globally named nitric oxide synthesis (NOS). NO can has both beneficial and harmful effects to the general pathophysiology of tissues. The controlled production of NO has important physiological roles in the mammalians nervous, immune and vascular systems.[10, 11] Whether NO is beneficial or harmful may

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systems.[10,11] Whether NO is beneficial or harmful may associated with NOS properties such as types of cells exposed to and producing NO, the chemical fate of NO, the amount of NO produced and the side of NO productions.[12]

NO is produced via different NOS. Moreover three different isoforms of NOS is known, which are endothelial NOS (eNOS), brain or neuronal NOS (nNOS) and inducible NOS (iNOS).[11, 13] Each isofom is a separate gene product in human; the gene for eNOS is located on human chromosome 7, the gene for nNOS is located on human chromosome 12 and the gene for iNOS is located on human chromosome 17.[14] The eNOS and nNOS produce low concentrations of NO during a small period of time. Also the major role of eNOS is related with vascular function and main role for nNOS can be related with retrograde signaling across synapses of neurons. Another isoform is iNOS, which identified in several cell types including macrophages and polymorphonuclear cells. iNOS is expressed in a response to inflammatory stimulation and produces large amounts of NO for a long time period.[15]

The human periodontal diseases are inflammatory disorders that give rise to tissue damage and loss, as a result of the complex interaction between pathogenic bacteria and the host's immune response, and also abnormal forces application on teeth can contribute to host tissue damage. During the development and progression of the diseases, there is increased colonization in the dental plaque of microorganisms termed periodontopathogens. Pathogens such as Gram-negative species, mobile rods and spirochetes may have the ability to invade gingival tissues. The interaction between pathogenic bacteria and the host's immune response are accompanied by an increase in cytokine expression and immunological activity in gingival tissues. During inflammatory condition, the inflammatory stimuli can induce NO production via activation of inflammatory cells such as macrophages and polymorphonuclear cells, which are already well established in the microenvironment of periodontal pathologies.[16] In addition to inflammatory condition, mechanical stress of abnormal forces, which foster tissues damage, may be related with mechanisms of NO production around the teeth.

NO appears to play beneficial effects including antimicrobial activity and immune modulation[17, 18] as well as detrimental effects including a cytotoxic action towards adjacent host tissues.[15, 19, 20] The presence of NO in periodontal disease may reflect the participation of an additional mediator of tissue damages responsible for disease progression. When NO is locally produced in high concentrations, it can act as a cytotoxic molecule, against infected cells, tumor cells and cells close to the production site,[17] and possibly resulting in tissue destruction.[15] It is currently known that NO has an important participation in bone metabolism regulation, directly acting over clastic cells.[21-24]

In this review, we discuss production of NO via different mechanisms during the inflammatory condition and abnormal force application in periodontal pathologies, and also relationship between NO or NO derived molecules and progression of periodontal disease.

Evidences of NO production in periodontal pathologies:

NO is a free radical that is an uncharged molecule with an unpaired electron, and produced by many cells with severe physiological and pathological conditions in the living organisms. NO is also secreted by inflammatory cells in the oral cavity.[25] Moreover, recent evidences have suggested that, high level of local NO production and periodontal pathologies can be associated with each other. In this part, it is discussed that, NO production in periodontal pathologies via inflammation and mechanical stress of force application, which are directly related with progression of periodontal pathologies.

Inflammation;

Periodontal inflammation can occur as a host's immune response against the bacterial pathogens, which are termed periodontopathogens such as Gram-negative species, mobile rods and spirochetes. The immune response in periodontal pathologies may be subdivided into two broad divisions, the innate (nonspecific) and the adaptive (specific) responses.[26] Innate reaction includes the inflammatory response but do not involve specific immune mechanism. Adaptive response tends to be more effective, as the host response is a specific immune response to the offending pathogens. The adaptive immune response can be subdivided into humoral and cell-mediated immunity. Antibodies mediate humoral immunity, whereas cell-mediated immunity involves the direct

action of immune cells such as macrophages, polymorphonuclear neutrophils (PMN), lymphocytes and host tissues cells. The immune response will result in further release of cytokines and proinflammatory mediators, which in turn will increase the inflammation and inflammatory cells, can produce a range of antimicrobial factors including reactive oxygen species (ROS) such as hydrogen peroxide, superoxide, hydroxyl radical and nitric oxide. These antimicrobial factors can be more harmful to the host.

During this complex inflammatory process, the presence of inflammatory stimuli can induce NO production by the inflammatory cells in the microenvironment of inflamed periodontal tissues. Especially, live bacteria in dental plaque such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum* play important roles in NO production by macrophages. Several studies indicated that, synthesis of NO via iNOS by macrophages, polymorphonuclear neutrophils (PMN) and lymphocytes is increased in periodontitis in mice[20] as well as by macrophages and endothelial cells in human periodontitis.[27] Also Batista et al.[16] demonstrated that, the number of iNOS cells is more elevated in periodontal pathologies than in clinically healthy gingival tissues, and that these iNOS cells are predominantly polymorphonuclear cells. The polymorphonuclear cells beside play major roles in defense mechanisms of the innate and adaptive responses.

In addition to inflammatory cells, the gingival fibroblasts[28, 29] and basal keratinocytes[30, 31] can express iNOS. The human gingival fibroblasts express iNOS, when stimulated by bacterial lipopolysaccharide (LPS) and interferon gamma (IFN-γ). The combination of LPS and IFN-γ has a potent effect to induce iNOS.[32, 33] However LPS or IFN-γ alone either suppresses the production of NO in fibroblasts.[34, 35] Induction of high production of NO by gingival fibroblasts may be related with healing process, and imbalance between tissue damage and tissue repair.[28] During gingivitis an early response involves the proliferation of the sulcular epithelium, possibly mediated by a differential proliferation and differentiation response of keratinocytes to NO.[36] Cytokine and bacterial toxin induced NO upregulates IL-1 stimulated IL-8 production by keratinocytes.[37]

On the other hand, some studies have suggested that polymorphonuclear leukocytes from patients with gingivitis and periodontitis suppress NO production and activity.[38, 39] Furthermore, same studies reported that, polymorphonuclear leukocytes isolated from gingival pockets have a stronger suppressive effect on NO production than polymorphonuclear leukocytes from venous blood. However, Aurer et al.40 reported that, it is not clear whether decreased NO in periodontal disease is simply a marker of increased inflammatory activity and tissue destruction or whether it is causative in the pathogenesis of the periodontal disease.

Application of force on tooth;

Application of force on tooth can be programmed such as during orthodontic tooth movement and non-programmed such as traumatic occlusal forces. Both of these types of force application produce a mechanical stress towards the periodontal tissues around the tooth. As a result of mechanical stress of force application, tooth movement can occur in dental arch. Also tooth movement involves cellular activation and responses in the periodontal tissues around the tooth.

During orthodontic tooth movement, remodeling which involves tissue damage and new tissue formation in the same time around the tooth alveolar bone,[40, 41] periodontal ligament[42, 43] and gingival tissues occur.[42] Moreover orthodontic tooth movement has similar properties with inflammation and wound healing. The cellular responses via application of mechanical force in periodontal tissues include cellular proliferation, migration, differentiation, and matrix synthesis and degradation.[41-43] The cellular response similarities between in periodontal tissues during orthodontic tooth movement and inflammation in periodontal pathologies can suggest that, NO production may be increased during orthodontic tooth movement.

In an experimental study in rats, Brogan et al.[45] searched relationship between localization of NO synthesis in periodontal tissues and orthodontic tooth movements, and reported those findings; a) The periodontal tissues of stationary rat molar teeth showed the presence of less eNOS and iNOS using immunohistochemistry, b) There are increased amounts of eNOS production after rat molar tooth movement in blood vessel walls, c) There is an increased amount of iNOS production after rat molar tooth movement in the periodontal ligament and

connective tissue between roots of moved teeth as well as around blood vessels, d) The presence of eNOS and iNOS was consistently seen in the odontoblast layer of pulp chambers of moved and unmoved teeth.[44]

Tooth movement also can occur via effects of mechanical stress of occlusal forces. The mechanical stress of occlusal force has been reported to be essential for the maintenance of periodontal ligament morphology and function. In the periodontal ligament, the mechanical stress of occlusal force may promote an increase in NOS expression. Animal studies shown that, occlusal force results in increased NOS expression and maintenance of periodontal ligament integrity in the rat periodontal ligament. Also NO may play an important role to mediate mechanical stress of oclusal force-induced changes in the rat periodontal ligament.[45]

Relationship between NO or NO derived molecules and the progression of periodontal diseases:

In previous part of our review, we discussed that, both inflammation, which occurs via complex interaction between pathogenic bacteria and host immune defense, and abnormal mechanical stress of orthodontic forces and occlusal forces induce NO production via especially iNOS and eNOS. However, the role of NO in periodontal health and disease is unclear, and there is little information about NO or NO derived molecules related direct and indirect effects during periodontal pathologies in previous studies. Despite the fact that, it is apparent that NO is a ubiquitous molecule and essential for many biologic functions such as host defense function and host tissue damaging, all of which are likely to be relevant to the pathogenesis of periodontal diseases.

Our aims in this part are thus, discussing the progression of periodontal diseases that can be related with presence of NO or NO derived molecules, discussing the properties of NO or NO derived molecules, these properties can be important in the host defense and host tissue damage during periodontal pathologies, and finally collection and discussion of information about relationship between NO or NO derived molecules mediated effects in periodontal tissues and progression of periodontal diseases.

Progression of periodontal diseases;

Periodontal diseases are known as an inflammatory response of gingival and surrounding connective tissue against the accumulations of bacteria or plaque on the teeth. Gingivitis describes ongoing gingival inflammation, without bone loss and attachment loss. Periodontitis is one of the most common destructive human inflammatory diseases, affecting the supporting tissues of teeth including gingival, bone and periodontal ligament. Gingivitis can become periodontitis when pathogenic bacteria persist and migrate below the tissue margin, and more extensive inflammation occurs, resulting irreversible destruction of the bone and connective tissues that support the teeth.[46]

The pathologic model for periodontal diseases begins with subgingival plaque infections, which is occurred by specific bacterial pathogens such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum*.[47] Previous studies related with progression of periodontal diseases indicated that, primary etiologic factor of periodontal diseases is microbial dental plaque, which include specific bacterial pathogens in its composition. Also periodontal diseases are fostered by other factors including abnormal mechanical stress of orthodontic forces and occlusal forces.[48] During progression of periodontal diseases, presence of both of these two etiologic factors result of NO production and also production of other reactive oxygen species as a host defense mechanism. If so, the detrimental effects of NO or NO derived molecules may play a role in progression of periodontal diseases.

The properties of NO or NO derived molecules;

Since NO is an uncharged molecule it is able to pass freely within cells, across between cells and biological membranes including mitochondrial membrane and cytoplasmic cell membrane.[49] The biological properties of NO is related with its unpaired electron, which makes NO a free and stable radical. NO is a lipophilic and high diffusible solute whose actions are dependent on both its concentration and form with in the cell.[50, 51] The easy penetration property of NO involves that, NO can effect all biologic mechanisms directly or indirectly in biologic organisms. NO has protective, regulatory and deleterious effects in tissues. These effects can occur

via direct effects including reaction with metal complex, interaction with metal-oxygen and oxo complex, and reaction with radical species, or indirect effects including reaction with oxygen molecule (O_2) and reaction with superoxide radicals (O_2).[50]

NO has reversibly activity on soluble guanylate cyclase[52] and inhibitor effect on cytochrome *c* oxidase[53] in nanomolar concentrations. Also NO has the potential to nitrosylate cysteine residues in proteins.[54] All physiological actions of NO occur in the nanomolar concentration range. Furthermore, in higher concentrations, NO can affect a wide variety of haem-containing and red/ox sensitive enzymes and ion channels.[14]

There are three major types of NO reactions with metals in biologic organisms: the direct reactions of NO with metal center, NO red/ox reaction with dioxygen metal complexes and high valent oxo complexes. NO react with a variety of metal complexes to form metal nitrosyls and with some translation metals to form stable metal nitrosyls complexes such as being Fe-NO complexes. An important direct effect of NO is the reaction between NO and oxyhemoglobin to form methemoglobin and nitrate.[55, 56]

The most relevant reactions of NO with metals in biological systems include hame proteins such as guanylate cyclase. Soluble guanylate cyclase is widespread and found in cells in every organ system.[57] NO binds to the haem moiety of the enzyme and increases its activity by 400-fold, catalyzing the conversion of guanosine triphosphate to cyclic guanosine monophosphate (cGMP). Elevation of c GMP relaxes smooth muscle in especially blood vessels inhibits platelet aggregation and adhesion, and blocks the adhesion of white cells to the blood vessel wall.[57] On the other hand, other enzymes such as haem containing enzymes, enzymes with Fe-S cluster including aconitase, NADH dehydrogenase and succinate dehydrogenase, the non-haem metalloenzymes, ribonucleotide reductase, and DNA itself become targets for NO action.[14] Also reaction between NO and enzymes may form a radical, for examples, when the catalytic turnover of ribonucleotide reductase reacts with NO, the tyrosyl radical species formed.[58, 59]

Indirect effects of NO are mediated by reactive nitric oxide species (RNOS). Indirect effects of NO via RNOS can be occur when NO reacts with oxygen molecules (O_2) or superoxide radical (O_2^-).[60] The interaction of NO with oxygen molecules leads to the formation of RNOS including nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3) and dinitrogen tetraoxide (N_2O_4).[50] These RNOS which derive from interaction of NO with oxygen molecules, are known to cause injury by further oxidative reactions with proteins in tissue. In many conditions the reaction of RNOS can induce cell death.

ROS mean a collective term encompassing free radicals including superoxide radical (O_2^-) and hydroxyl radical (OH^-) species, as well as non-radical oxygen derivatives such as hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCL). Both inflammatory cell-derived ROS and NO or NO derived molecules have important beneficial and detrimental roles. Under conditions of stress that resemble certain inflammatory and pathological conditions, NO can react with superoxide radical (O_2^-) to form a radical molecule, named peroxynitrite ($ONOO^-$). It has been speculated that $ONOO^-$ formation is a primary pathways of NO metabolism.[61, 62] The two major indicators about formation of peroxynitrite are that, first, the relative amount of NO and superoxide is produced and second, the biological reaction of these radicals with other biological components is limited.[63] Peroxynitrite is a potent, nucleophilic RNOS that readily nitrates phenol-containing compounds such as tyrosine residues in proteins. Nitration of tyrosine residues can regulate protein function by inhibiting tyrosine phosphorylation.[64] Peroxynitrite can also induce DNA damage, lipid peroxidation, protein degradation, and *S*-nitrosylation of cysteine residues in proteins that can lead to cell damage and often death.[65, 66]

Effects of NO or NO derived molecules in periodontal pathologies;

Antibacterial effect;

Induction of expression of iNOS is thought to be an important host defense mechanism that can lead to death of certain pathogens. However, not all bacteria are sensitive to NO. For example, whilst the growth of *Staphylococcus aureus* is potently inhibited by activation of NO, *Salmonella typhimurium*, *Escherichia coli* and *Listeria monocytogenes* appear resistant.[67] During progression of periodontal pathologies some bacterial species such as

Porphyromonas gingivalis and *Fusobacterium nucleatum* in composition of dental plaque play major role via invading gingival tissue. The iNOS-generated NO is an important element of the host defenses against these pathogens, because experimental studies shown that, the iNOS-generated NO is able to kill *Porphyromonas gingivalis*[68] and *Fusobacterium nucleatum.*[69]

Salivary glands contain nitric oxide synthetase, also NO is important in salivary vasoregulation and secretion.[70] NO is formed in oral cavity of health individuals and its concentration is directly related to salivary nitrite,[71] which in turn is associated to dietary nitrate intake, and intraoral pH level.[72] It has been shown that an intraoral decrease in pH is associated with an increase in the generation of NO.[72] However, some factors in saliva suppress the synthesis NO or destroy already produced NO. The most important molecule, which suppresses NO productions, is arginase in saliva. Arginase, which is an arginine-depleting enzyme, can compete with NOS for the common substrate L- arginine, and thus inhibit NO production. Ozmeric et al.[74] searched the salivary arginase activity in patients with periodontitis They detected that; the patients with perodontitis have higher salivary arginase activity in oral cavity when compared to periodontally healthy individuals.[73]

The most important beneficial effect of NO in inflammatory conditions is antimicrobial activity against certain bacterial infections.[74] The controlled production of NO can contribute host defenses mechanism via killing bacterial pathogens in the periodontal microenvironment. The increased salivary arginase activity in periodontitis may cause decreasing of NO production under the normal level, and also leads to a decrease in the antibacterial property of saliva and cause periodontal tissue to become more susceptible to existing periodontal pathogens.

Host tissue damaging effect;

The most common damaging effect of NO on host tissues in periodontal pathologies is associated with inducible isoform of cyclooxygenase (COX-2) enzyme. Expression of COX-2 is increased predominantly in fibroblasts, and in resident and free macrophages, but not in PMN in periodontitis, and also these cell types are the source of the prostaglandins (PGs) produced by COX-2.[75] Recently, in vitro studies shown that, COX-2 founds in periodontal ligament cells in response to mechanical stress of abnormal forces[76] and in the oral keratinocytes.[77] In the healthy gingivomucosal tissues, low COX-2 activity and low or non-detectable prostaglandin E_2 (PGE_2) levels are found in periodontal tissues and crevicular fluid of normal healthy subjects.[76] However, during the progression of periodontal disease the PGE_2 levels increase related with periodontal status and number of COX-2 immunoreactive cells was elevated approximately 5-fold one week after the induction of periodontitis.[76]

It has confirmed that, PGE_2 levels, which depend on activity of COX-2, within periodontal tissues and crevicular fluid, are highly correlated with periodontal tissue destruction. In periodontitis COX-2 activity may be induced by bacterial endotoxin, proinflammatory cytokines and probably NO or peroxynitrite, because the iron-heme center of COX may be a potential target for NO, and peroxynitrite is a potential substrate for the peroxidase activity of COX.[78-80] Also some COX products can play a role for NO pathway, but this role is not major.[80] On the other hand, in peroxynitrite formation, COX-2 may act as a source of superoxide.[81] In addition, NO mediated or peroxynitrite mediated increase in the production of proinflammatory PGs may provide a linkage among NO, reactive oxygen species and lipid mediators of inflammation.[20, 78-80]

ROS are active in depolymerization of extracellular matrix components, lipid peroxidation (LPO), oxidation of enzymes such as anti–proteases, increased apoptosis in deepest area of the sulcular pocket, induction of pro–inflammatory cytokines and DNA damage.[82] The activation of neutrophil pro-collagenase (MMP-8) by NO derived reactive molecules such as peroxynitrite and nitrogene dioxide may be a mechanism by which ROS production may contribute to the profound early loss of collagen in gingival lesion.[83] In fact, NO exhibits anti-proliferative effects and low level of NO is necessary for collagen synthesis but overproduction of NO can be harmful for collagen synthesis. Previous studies in the rodent model shown that, NO are known to potentiate matrix degradation via suppression of proteoglycan synthesis and collagen synthesis,[84] and also via upregulation of metalloproteinase activity.[85]

In bone metabolism, NO appears to be an important molecule. The remodeling of bone is

characterized by osteoblast-mediated bone formation and osteoclast-mediated bone resorption. Both of osteoblast and osteoclast can produce and response to NO molecule.[86, 87] NO has biphasic effects on osteoblast, high concentrations of NO are inhibitory for osteoblast, while low concentrations of NO stimulate bone formation by inducing proliferation and differentiation of osteoblast.[88-90] However, it has been difficult to predict whether overproduction of NO during inflammation increases bone loss or prevents bone loss in periodontal diseases.

On the other hand, in many cell types such as macrophages, pancreatic islets, neurons and thymocytes, nitric oxide, which is a form of ROS, activates apoptosis.[91] Also induction of apoptosis may seen as a response to oxidative DNA damage, this damage can occurred by ROS, and especially by NO. The induction of NO synthesis expression may also inhibit fibroblast proliferation and induce apoptosis, contributing to the imbalance of tissue destruction with tissue repair that is characteristic of periodontitis. A study of relationship between apoptosis and destructive inflammatory periodontal disease showed that, there is more proliferation than apoptosis in sulcular portion of periodontal epithelium except deepest part of the sulcular epithelium.[92] More keratinocytes undergo apoptosis in the deepest area of the sulcular pocket closest to the junctional epithelium in destructive form of inflammatory periodontal tissues.

In addition, periapical area of tooth can be affected from bacterial pathogens during progression of pulpal infection. Periapical periodontitis can occur in this area. Suzuki et al.[94] demonstrated that, macrophages and lymphocytes express iNOS in granulation tissue of most periapical inflammatory lesions. The iNOS expression and consequent NO productions are considered this mediating periapical tissue destruction, and causing chronic inflammation and lesion expansion.

According to these evidences about NO or NO derived molecules associated tissue damage, local overproduction of NO or peroxynitrite may destroy not only the invading bacterial pathogens, which are primary etiologic factor for progression of periodontal diseases but also periodontal tissues via these complex mechanisms. Also these evidences suggest that elevated NO production could be important in the pathogenesis of periodontal diseases. However, it is unknown that, NO acts to cause injury or protection under which conditions. Further studies will be continuous to clarify the relationship between NO and periodontal diseases.

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