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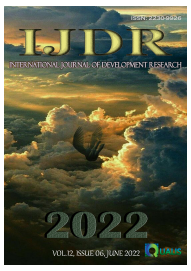

RESEARCH ARTICLE **OPEN ACCESS**

MECHANISMS OF THE IMMUNE/INFLAMMATORY RESPONSES AND PRODUCTION OF REACTIVE OXYGEN AND NITROGEN SPECIES IN GINGIVITIS AND PHARMACODYNAMICS OF ANTI-INFLAMMATORY DRUGS

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ABSTRACT

Introduction: Gingivitis is characterized as an inflammation of the gum and can have different etiologies, however, most cases occur due to poor oral hygiene and accumulation of bacteria in dental plaque, causing periodontal disease, which can progress to tooth loss. Knowing the pathophysiology of gingivitis is necessary for an effective therapeutic intervention by the professional. This work aims to describe the pathophysiological process of gingivitis and discuss the mechanism of action of anti-inflammatory drugs. **Methodology:** This work is a literature review carried out on Google Scholar, PUBMED and SciELO platforms. **Results and Discussion:** Bacteria accumulated in the plaque are recognized by phagocytes, which are activated and induce immune and inflammatory responses, in addition to activating oxidative and nitrosative stress pathways, resulting in bacterial death and gingival necrosis. In the face of aggression, gingiva cells activate the arachidonic acid cascade, which gives rise to several pro-inflammatory mediators; Typical anti-inflammatory drugs block enzymes in this cascade, inhibiting the production of these chemical signals. **Conclusion:** Therefore, immune and inflammatory responses are important for the prognosis of gingivitis, however, in some cases, therapeutic interventions are necessary so that there is no great tissue and tooth loss.

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INTRODUCTION

The oral mucosa is a membrane lining within the oral cavity, divided into three distinct zones: masticatory mucosa (covers the gingiva and hard palate), specialized mucosa (dorsum of the tongue) andlining mucosa (oral mucosal membrane). Healthy gingiva covers the alveolar bone and the dental root atthe level of the cement-enamel junction (CEJ), with a color ranging from light red to purple, and aspeckled or "orange peel" appearance, as it is clinically known (BHATIA, *et al*., 2015).The gingival tissue is subjected to constant mechanical and bacterial aggressions that trigger local inflammatory responses, causing pain, discomfort and bleeding. There are several stimuli that can generate inflammation in gingiva, among them, chemical processes (irritating chemical substances), physical/ mechanical (burns, cuts, non-carious cervical lesions) and biological (for example, periodontal disease caused by bacterial infection associated with dental plaque) (KURGAN and KANTARCI *et al*., 2018). Research in paleontology affirms that gingival diseases afflict human beings since the beginning and that historical records from different cultures reveal awareness of periodontal disease and the need for treatment, occupying a significant space in these historical records, suggesting this disease as one of the most common pathological conditions found. Periodontal disease is associated with several pathological conditions, such as inflammation of the gingiva and degeneration of the periodontal ligament, cementum and loss of alveolar bone. In the current classification of periodontal diseases, in force since 2018, there are three major groups: 1) periodontal health, gingival conditions and diseases (which includes gingivitis); 2) Periodontitis; and 3) other conditions that affect the periodontium (STEFFENS *et al*., 2018). Among these diseases, gingivitis stands

out, which is characterized by the inflammatory response triggered by the presence of bacteria at the margin and also throughout the entire remaining gingival unit. These bacteria can usually colonize several oral anatomical sites, that is, they are found adhered to the teeth, gingival sulcus or close to it. It is important to emphasize that this inflammatory response in the gingiva is crucial for the amplification of the immune response (activation of leukocytes) with subsequent microbiological clearance (BHATIA *et al*., 2015; KURGAN and KANTARCI *et al*., 2018). Despite the benefits, the immune and inflammatory responses, when amplified, produce a series of molecules toxic to the gingival tissue, such as reactive oxygen and nitrogen species (ROS and ERN, respectively), which act by fighting pathogens and also causing injuries to host cells, inducing necrosis and tissue loss. For the patient, several complications can arise from these processes, such as: Presence of bacterial plaque, tissue loss, edema, bleeding, sensitivity and increased gingival exudate. In this context, to try to reverse the process, it is important to ensure the complete removal of bacterial plaque, so that the inflammatory stimulus is stopped, changing the pattern of the gingival immune response to anti- inflammatory, with posterior activation of the healing process (TÓTHOVÁ and CELEC, 2017). Taking into account the clinical importance of this disease in the course of human evolution and until the present day, it is necessary to understand the entire gingival inflammatory process, from the resulting tissue destruction to the mechanisms of pharmacological intervention established by the dentist. Thus, this article aims to describe the pathophysiological process of gingivitis and to correlate it with oxidative and nitrosative stresses, which act as agents of tissue aggression; as well as addressing the anti- inflammatory mechanisms of the most prescribed drugs for this clinical condition, when necessary.

METHODOLOGY

This article is a descriptive literature review. For this, a bibliographic survey of articles was carried out, searched in portuguese and english, using the following descriptors: Gingivitis, immune, inflammatory, response, oxidative, nitrosative, stress, on Scielo, Google Scholar and PubMed databases, for later review summary. It was selected a total of 34 articles published between the years 2014 and 2021, from high-impact journals with approaches on the subject. Then, 48 articles with repetitive, outdated topics and published in journals with a lower impact factor were excluded. In this sense, the text was produced following a sequence of events on gingival tissue and the role of oxidative and nitrosative stresses in the pathophysiology of gingivitis, focusing on tissue loss, its mechanisms and the drugs prescribed by dental surgeons for the treatment of cases.

RESULTS AND DISCUSSION

Anatomy and Physiology of the Gum: Gingiva is the part of the masticatory mucosa that covers the alveolar bones of the mandible and maxilla and surrounds the cervical portion of the teeth. Formed by vascularized and innervated, fibrous epithelial tissue and an underlying connective tissue called lamina propria, which when healthy has a color ranging from pink to soft coral. Anatomically, among other functions, gingiva play roles in sealing the spaces between the teeth, acting in the prevention of the development of bacterial plaque, which can lead to health problems; still, in general terms, it can be classified into marginal gingiva, attached gingiva, and intermediate parts (figure 1A), each one presenting specific functions in dental physiology (BHATIA *et al.,* 2015). The marginal gingiva or free gingiva has a pink color, an opaque surface and a firm consistency. It comprises the gingival tissue of the buccal and lingual or palatal parts of the teeth, in addition to the interdental gingiva or interdental papillae, which act mainly to protect the teeth and limit the growth of bacterial plaque, which can lead to periodontitis. It extends from the gingival margin to the cement-enamel junction (CEJ), as shown in Figure 1B. Also, in the marginal gingiva we find the gingival sulcus that completely surrounds the tooth, delimiting this space. In healthy gingiva, the gingival sulcus has a depth that varies from 0.5 to 2 mm (GÓMEZ-POLO *et al*., 2019).

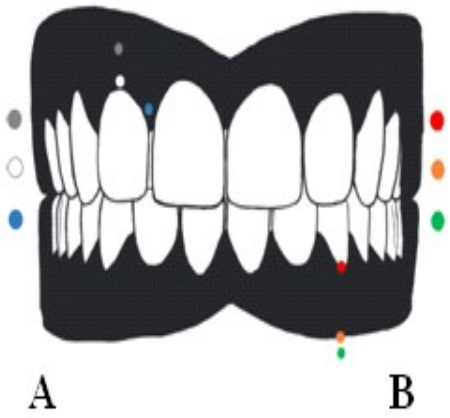


Figure 1. Basic anatomy of the gingiva and related anatomical regions. Side A: AG = Attached Gingiva; MG = Marginal Gingiva; IG = Interdental Gingiva. Side B: MGJ = Mucogingival Junction; CEJ = Cement-enamel Junction; AM = Alveolar Mucosa

On the other hand, the attached gingiva (Figure 1B) is a firmer, pinkish opaque structure, which has a speckled texture, an important feature for pathological processes, since this feature tends to disappear in inflammation processes, serving as a biological marker for edema, which is the leakage of fluid from the vessels into the tissue. This structure is present just after the marginal gingiva and has a characteristic continuous to it, in addition, it is directly connected to the periosteum of the alveolar bone and extends to the mobile alveolar mucosa, being demarcated by the mucogingival junction (figure 1B). This structure is formed mainly of dense collagen fibers and performs a variety of functions, among them, the attachment and insertion of the gingiva to the cement of the underlying alveolar bones, in addition to assisting in the nutrition and perfusion of dental tissues. The interdental gingiva is the tissue responsible for the formation of interdental papillae, which may have a pyramidal or "col" shape. Furthermore, it occupies the gingival embrasure and is determined by the contact relationships between the teeth, by the width of the proximal surface of the teeth and the cement-enamel junction (HYUN *et al*., 2017). Knowing the basic anatomy and physiology of the gingiva is enough to be aware of the importance of this tissue for oral health in general, however, there are several problems that affect the gingival tissue, usually triggering immune and inflammatory responses *in situ*, compromising the natural physiology of the tissue and causing pain and discomfort to the patient. Among the main problems related to gum health, it can be mentioned periodontitis, abfraction and lesions resulting from biological (microorganisms), chemical (irritating chemical substances), physical/mechanical (burns, cuts), among others (HYUN *et al*., 2017; KURGAN and KANTARCI, 2018).

Mechanisms of inflammation and pathophysiology of gingivitis: Immune and inflammatory responses are part of the common pathogenesis of several diseases, including the stomatognathic system, such as gingivitis. Therefore, it consists of a gingival tissue response against bacterial antigens present mainly in dental plaque. These antigens, which can be microbial toxins, are recognized by cells of the immune system, activating them and generating a local inflammatory response. This process, although diversified due to the great variation of the antigenic molecules, generally follows a sequence of common events that generally are: 1) Recognition of harmful stimuli (eg, microbiological antigens) through receptors present on the leukocyte membrane, such as toll-like receptors (TLR), which act by binding to these antigens, generating cellular activity; 2) Activation of immune system cells, ie, inducing the proliferation and differentiation of leukocytes, as well as the chemotaxis (migration) of these cells from the vessels to the gingival tissue; 3) Production and release of inflammatory mediators, such as chemokines, which cause

the recruitment of leukocytes from the vessels to the tissue, and cytokines, responsible for the activation of these cells; 4) Activation of free radical production pathways, such as oxidative and nitrosative stresses; and 5) Production of reactive oxygen species (ROS) and nitrogen (RNS) in excess, which leads to the death of the pathogen and also injuring gum cells (CHEN *et al*., 2018; KURGAN and KANTARCI *et al*., 2018).

The process of gingival inflammation usually starts with the formation of bacterial plaque, an acquired film composed mainly of glycoproteins and antibodies, which resists salivary flow and supports the adhesion of bacteria and the beginning of an infectious process. Initially, Gram-positive facultative microorganisms colonize the medium and adhere by means of adhesins, which interact with specific receptors present in the dental pellicle. Accordingly, in secondary colonization, the transition to Gram-negative microorganisms occurs, which adhere to pre-existing bacteria in the bacterial plaque, producing substances toxic to the host tissue; It is important to note that Gram-negative bacteria are known to have lipopolysaccharide (LPS) as a structural substance of the cell wall, which contributes to increased inflammation, since it is a highly immunogenic molecule that amplifies the immune and inflammatory response. through activation of leukocytes (phagocytes) via TLR receptor (TRL4 subtype) (Antonini,*et al*., 2014). The aggression caused to the gingival tissue induces a tissue response against the etiologic agent, activating the arachidonic acid cascade, responsible for the production of the main pro-inflammatory mediators, such as protaglandins, prostacyclins, thromboxanes and leukotrienes (NĘDZI-GÓRA *et al*., 2017; TALLIMA and RIDI, 2018). In this sense, phospholipase A2 enzyme acts on the lipids of gingival tissue cells, having as substrate the fatty acids of the phospholipids molecules of the plasma membranes of gingival cells, hydrolyzing the bond between the side chains of the molecule and the glycerol portion, releasing arachidonic and lysophosphatidic acid to the medium, as seen in figure 2. Arachidonic acid, in turn, is substrate for two enzymes that form different inflammatory mediators, the cyclooxygenases (COX) and lipoxygenases (LOX). When arachidonic acid undergoes the action of LOX, it mainly produces leukotrienes (figure 2), which are lipids of the eicosanoid family and have different biological functions, among them, some subtypes allow the chemotaxis of leukocytes to the affected tissue by increasing vascular permeability; these leukotrienes are important for infiltration of cells, so they are pharmacological targets for several drugs in clinical routine e research. In order to balance chemotaxis, LOX also produces lipoxins, that act by inhibiting chemotaxis (ÖNDER *et al*., 2017).

In the pathophysiology of gingivitis, it is important to emphasize that COX-derived mediators are more important, with regard to the pharmacological issue. In this context, when arachidonic acid undergoes the action of COX, the production of prostanoid mediators occurs, which are classified into the following families of molecules: Prostaglandins, prostacyclins and thromboxanes (Figure 2), which are inflammatory mediators, of which some subtypes are important and linked to the pathophysiology of gingivitis. It is important to remember that these molecules have several subtypes, whose functions are widely diversified in the organism; here, it was listed only functions related with the inflammatory process. In summary: COX-2 oxidizes arachidonic acid, forming the prostaglandins PGG2 and PGH2, which give rise to prostanoids. Prostaglandins, mainly PGD2 and PGE2, signal via receptors the processes of vasodilation and increase in vascular permeability, contributing to formation of edema. Thromboxanes, especially TXA2 subtype, act by promoting vasoconstriction and platelet aggregation in hemorrhagic processes, preventing blood loss. On the other hand, prostacyclins act mainly by counterbalancing the action of TXA2, exhibiting vasodilator effect and inhibition of platelet aggregation. Complementarily, these prostanoids are directly related and linked to the signaling of the five classic signs of inflammation, that are heat, redness, swelling, pain and loss of function (ÖNDER *et al*., 2017; STEFENS *et al*., 2018).

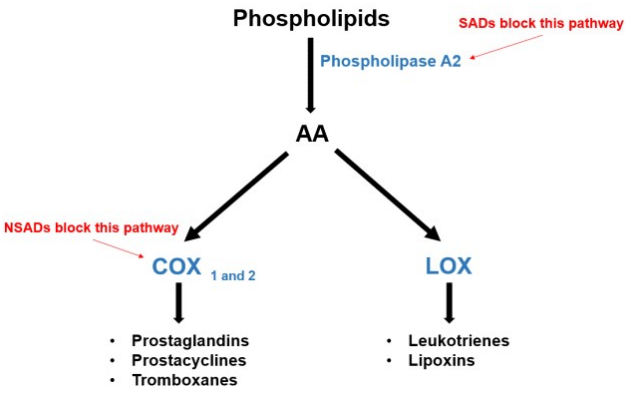


Figure 2. Enzyme phospholipase A2 acts on membrane phospholipids to form arachidonic acid. This, in turn, undergoes the action of COX and LOX enzymes. LOX produces leukotrienes and lipoxins; COX is responsible for the production of prostaglandins, prostacyclins and thromboxanes. The red arrows indicate the mechanism of action of non-steroidal anti-inflammatory drugs (NSADs) and steroidal anti-inflammatory drugs (SADs), in which they act by inhibiting these enzymes

Pharmacology of inflammation and mechanism of action of anti-inflammatory drugs: The treatment of gingivitis linked to infectious processes is routinely performed by dental surgeons in offices mainly through the surgical removal of bacterial plaque, not requiring pharmacological treatment in most cases. However, in more severe cases of gingivitis, with regard to anti-inflammatory medicines, non-steroidal anti-inflammatory drugs (NSADs) are basically prescribed. Even so, there are other important classes of these medicines, such as steroidal anti-inflammatory drugs (SADs), which act by inhibiting phospholipase A2. NSAIDs act basically by inhibiting cyclooxygenases (COX), of which they have three isoforms with different functions: COX-1, involved in several physiological processes; COX-2, the main isoform involved in inflammatory processes; and COX-3, recently discovered, whose physiological/pathophysiological roles are still unclear (CAMPOS *et al*., 2017; GUGLIANDOLO *et al*., 2019). NSADs are indicated to act by inhibiting COXs (in particular COX-1 and COX-2), resulting in non-production of prostaglandins, prostacyclines and tromboxanes, as also shown in figure 2 (red arrow). In this case, it is important to remember that several adverse effects of NSAIDs are linked to the blockade of COX-1, which plays an important role in the protection of the gastric mucosa of the stomach and also in the regulation of renal function; thus, the use of non-selective NSAIDs is linked to renal dysfunction and gastrointestinal bleeding, contributing to the development of gastritis and ulcers (FREITAS *et al*., 2019).

In an attempt to reduce the adverse effects resulting from COX-1 blockade, classes of drugs with selective inhibition of COX-2 were developed, drugs called COXIBs, which include celocoxib, etoricoxib, lumiracoxib, parecoxib, rofecoxib, valdecoxib, among others. However, despite avoiding several adverse effects, selective blockade of COX isoform 2 has already been shown to produce serious adverse effects, such as thrombus formation and cardiovascular events, such as increased blood pressure and the risk of myocardial infarction and cardiac insufficiency; It is worth remembering that SADs, discussed below, are also related to renal failure, so they are also used only in rare and specific cases with regard to the treatment of oral health problems (PADOIN *et al*., 2018). SADs have the mechanism of action by blocking the phospholipase A2 enzyme, preventing the formation of arachidonic acid (figure 2, red arrow), therefore, presenting more potent effects than NSAIDs, since it blocks both COX and LOX pathways. The use of these medicines is practically not part of the dental prescription routine, mainly because these drugs are related to more serious adverse reactions than NSAIDs, such as fluid retention and even immunosuppression (FREITAS *et al*., 2019). A summary of the main NSADs (selective and non-selective) and SADs, as well as their mechanisms of action are listed in table 1.

Immune response against dental plaque bacteria: It is important to remember that inflammation is a tissue response, therefore, any organic tissue can induce its own inflammation through the arachidonic acid cascade, when injured.

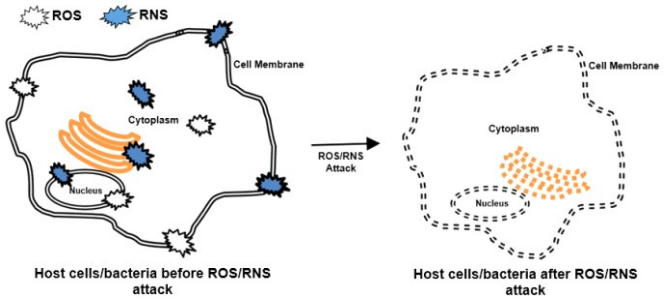
Table 1. Main NSAIDs and SADs available and their mechanisms of action. In cases of gingivitis, when pharmacological intervention is required, the drugs recommended for standard treatment are non-selective NSADs

NSADs: Inhibition of COXs		SADs: Inhibition of phospholipase A2
Non-selective: Inhibits COX 1/2	Selective: Inhibits COX-2	
Acetylsalicylic Acid (ASA)	Celocoxib	Betamethasone
Ketoprofen	Etoricoxib	Dexamethasone
Diclofenac	Lumiracoxib	Hydrocortisone
Ibuprofen	Parecoxib	Prednisone
Meloxicam	Rofecoxib	Prednisolone
Nimesulide	Valdecoxib	Triamcinolone Acetonide
Piroxicam		

The inflammatory process can also be originated and amplified with the activation of the host's immune response, that is, the activation of several leukocyte types. The presence of bacterial plaque causes the activation of tissue-resident immune cells, such as macrophages and dendritic cells through the recognition of antigens (bacterial molecules) by membrane surface receptors, with TLRs being one of the main families involved in the recognition of these molecules by the phagocytes (CHEN *et al*., 2018). After binding the antigens to the receptors, phagocytes are activated and produce pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1), which are inductors of the inflammatory response and also of the phagocytosis process. Thus, these cells internalize the bacteria, which remain alive inside vesicles called phagosomes; these phagosomes fuse with other cytoplasmic vesicles called lysosomes, which are rich in enzymes responsible for producing free radicals.

The fusion of the phagosome and lysosome forms another structure called phagolysosome, which is responsible for activating ROS and RNS-producing enzymes; these reactive species act on microbiological degradation and this whole process is necessary for the presentation of antigens through the exposure of microbial molecules externalized by the major histocompatibility complex II (MHC-II) on the surfaces of macrophages and dendritic cells, thus, these antigens can be presented to helper T lymphocytes (express CD4 glycoprotein) via T cell receptors (TCR); this lymphocytic lineage is essential for the immunological signaling of effector, humoral and memory responses to occur, through the activation of cytotoxic T lymphocytes (which express CD8 glycoprotein) and B lymphocytes, that differentiate into memory cells and plasma cells, the latter being responsible for active antibodies production (DUTZAN *et al*., 2016; CAMPOS *et al*., 2017). Regarding the functions of helper T lymphocytes ($CD4^+$), after activation, these cells mainly produce different cytokines whose combinations result in different signaling, such as pro and anti-inflammatory responses. Cytokines modulate the inflammation by activating or inhibiting the immune response. These immune and inflammatory responses last and are amplified while there are still antigens or antigenic molecules in the gingival tissue, which induces the Th1 response pattern (pro-inflammatory), important for bacterial clearance. After death and cleaning of pathogens, auxiliary lymphocytes change the cytokine pattern to Th2 type (anti-inflammatory), also resulting in the alternative activation of M1 to M2 macrophages, in order to enable the healing processes (WU *et al*., 2015; DUTZAN *et al*., 2016). Anti-inflammatory drugs that act by inhibiting the action of cytokines are less common in clinical practice and are still highly studied experimentally. However, the dosage of these molecules in gingival tissues is important for studying new drugs with anti-inflammatory activity in pre-clinical studies (DUTZAN *et al*., 2016; CAMPOS *et al*., 2017). The activation of the immune response is also an important pathway for production of free radicals and reactive oxygen and nitrogen species.

Free radicals and reactive oxygen and nitrogen species: During an infectious process, such as periodontitis, phagocytes residing in gingiva are activated and initiate the process of phagocytosis, as discussed earlier.



It has already been shown that the phagocytosis of microorganisms acts as one of the main triggers for the production of reactive oxygen and nitrogen species, which are rich sources of free radicals and oxidation and nitrosation molecules of biological compounds. Excess production of free radicals caused mainly by phagocytosis of pathogens is a process resulting from oxidative and nitrosative stresses, in which the biological antioxidant system presents a deficit in relation to the amount of reactive species produced, becoming unbalanced and contributing to the tissue damage (MOURA *et al*., 2017; TÓTHOVÁ and CELEC, 2017). It is important to emphasize that the production of free radicals is extremely important for immune defenses, since they are microbicidal molecules, related to the elimination of pathogens from the host. Several cells produce free radicals, however, in periodontitis, phagocytes are the main sources of these molecules, whose production is increased by the release of pro-inflammatory cytokines initially produced by phagocytes, later by phagocytes and activated lymphocytes (WU, *et al*., 2015; Leewananthawet, *et al*., 2019). In bacterial periodontitis, polymorphonuclear (PMNs) cells, such as neutrophils, are important producers of free radicals in innate immunity (WANG *et al*., 2017).

Atoms and molecules tend to be stable when their electrons are arranged in pairs, especially with regard to the last electron shell. These electrons, despite having a negative charge, are able to form pairs through their spins, which are angular behaviors of these subparticles already associated with rotations in different directions, causing the annulment of their repulsion forces and consequently forming pairs and stabilizing atoms. When an atom loses or gains electrons, it becomes a free radical. These radicals try to stabilize themselves by attacking biological molecules close to them, getting stability, however, creating new free radicals by sequestering electrons from atoms of biomolecules, generating a biological cycle of production, with microbiological and tissue destruction (DA SILVA *et al*., 2018). These radicals act by killing the bacteria present in the gum, however, they also attack cells of adjacent tissues, causing tissue damage. The main molecules attacked by free radicals are membrane compounds (bacterial and gum cells), such as proteins and phospholipids; In addition, free radicals also react with compounds that form nucleic acids (NGUYEN *et al*., 2016). The action of free radicals and oxidation and nitration molecules with biological targets can be seen in Figure 3.

Reactive nitrogen species: In a case of periodontitis, whose etiology is mainly bacterial, nitric oxide (NO) synthesis pathway is activated in response to bacterial antigens, a gas with several biological functions (physiological and pathophysiological), presenting roles in the immune and inflammatory pathways, with action on the vascular response and as a pro-inflammatory signal; in addition to being used as substrate for production of free radicals and oxidation and nitrosation molecules (SCAREL-CAMINAGA *et al*., 2017). NO production is mediated by the activity of nitric oxide synthase (NOS) enzymes, whose substrate is the amino acid L-arginine. NOS produces NO through two reactions, the first is by hydroxylation of

one of the guanidide nitrogens of L-arginine, forming N^G-hydroxyl-L-arginine (L-NHA), in a reaction involving the nicotinamide adenine dinucleotide phosphate (NADPH) enzymatic complex and oxygen (O_2); and the second step, where L-NHA is biotransformed into nitric oxide (NO^X) and L-citrulline by the same reaction scheme described in step one, as can be seen in figure 2 (MOURA *et al*., 2017; SCAREL-CAMINAGA *et al*., 2017).

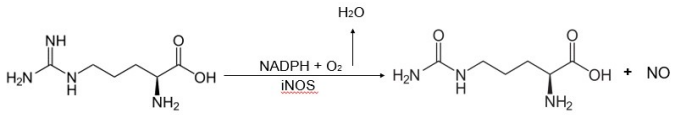


Figura 4. Formation of NO in cases of gingivitis from L-arginine through the action of the enzymes iNOS and NADPH, resulting in the formation of L-citrulline and nitric oxide

NO is a free radical gas that plays a diverse role in several physiological phenomena, such as the regulation of vascular tone, acting as a vasodilator in various tissues. For this reason, NOS has three isoforms, activated according to physiological or pathophysiological needs, as in inflammation (MEIRELLES *et al*., 2016; NAGARKOTI et la., 2019). Of the three NOS isoforms, there are two constitutive (cNOS) that are sensitive to calcium (Ca++) and calmodulin, the endothelial (eNOS) and the neuronal (nNOS) ones, which are responsible for the production of NO with roles mainly in cell signaling in these tissues; and an inducible isoform (iNOS), mainly related to the inflammatory production of NO, as in bacterial periodontitis, acting as free radical and also as signaling molecule of the immune and inflammatory responses, amplifying the process until the bacterial clearance of the dental plaque occurs (ÖZDEMIR *et al*., 2016). iNOS is present in macrophages and activated dendritic cells and unlike the others, it is Ca^{++}-independent, its activity is induced by the release of cytokines, such as TNF-α, IL-1 and interferon-gamma (IFN-γ), whose syntheses are increased during gingivitis caused by periodontitis; the presence of antigenic molecules, derived from bacteria (and other pathogens) is also capable of activating iNOS in these phagocytes. It is important to remember that RNS production continues until complete depletion of L-arginine or death of the pathogen (CINELLI *et al*., 2020). In inflamed gingival tissue, NO can give rise to several other reactive species, such as the formation of peroxynitrite ($ONOO^-$), which occurs through the reaction of NO^x with the superoxide anion (discussed in the next chapter). This compound is associated with increased tissue damage through oxidation and nitration of the same biomolecules described above, causing an increase in the rate of lipid peroxidation, a biomarker to measure the level of tissue loss/injury. Furthermore, $ONOO^-$ can react with protons (H^+) of the medium, giving rise to the hydroxyl radical (OH^-), potentiating the oxidative damage produced by ROS and RNS (ISLAM *et al*., 2015).

Reactive oxygen species in gingivitis: During gingivitis, mainly resulting from an infectious process, the main microbicidal molecules produced are the reactive oxygen species (ROS) which, when secreted in excess, generate the oxidative stress, responsible for bacterial death and tissue injury. ROS are made up of free radicals and other unstable molecules, capable of forming free radicals when attacking other biological compounds. The major free radicals formed in inflammation are the superoxide anion ($O2^-$), hydroxyl (OH^-), peroxyl (ROO^-), alkoxyl (RO^-), among others; and non-radical ROS, such as hydrogen peroxide (H_2O_2), organic hydroperoxides (ROOH), singlet oxygen (O_2^-), excited carbonyls (RCO) and others. Furthermore, ROS can react with RNS and form free radicals with high oxidation and nitration capacity, such as peroxynitrite ($ONOO^-$) and related molecules (SIES, *et al*., 2017; CHERIAN *et al*., 2019). A summary of the EROs can be seen in the Table 2. It is important to remember that cell death mechanisms are similar for all these reactive species, both radicals and non-radicals. For this reason, this section will only address the main molecules of the oxidative stress, from which the others are derived. ROS can be formed in physiological processes such as through the mitochondrial respiratory chain of gingival fibroblasts and in inflammatory processes, as in case of periodontal disease-induced gingivitis.

Table 2. Classification of the main reactive oxygen species (ROS) into free radicals and non-radicals. Peroxynitrite is a free radical derived from the combination of ROS and RNS, so it was not included in the table

Free Radical ROS	Non-free radical ROS
Superoxide anion (O_2^-)	Hydrogen peroxide (H_2O_2)
Hydroxyl Radical (OH^-)	Organic hydroperoxide (ROOH)
Peroxyl radical (ROO^-)	Singlet molecular oxygen
Alkoxyl radical (RO^-)	Excited carbonyls (RCO)
	Ozone (O_3)

The enzymes involved in the production of these compounds are NADPH oxidase (NOX), xanthine oxidase (XO), myeloperoxidase (MPO), COX and LOX, which basically give rise to O2.-, one of the most potent radicals and precursor of other ROS (CHERIAN *et al*., 2019; CORRÊA *et al*., 2019). Below it will be addressed its production via NADPH oxidase, one of the major enzymes for oxidative processes. In gingivitis, when there is recognition and phagocytosis of antigens by phagocytes, such as neutrophils, macrophages and dendritic cells, there is also a trigger for the formation of ROS via the activity of different enzymes. Through the NADPH oxidase pathway, as shown in figure 5, this enzyme acts as an electron donor to molecular O_2, reducing this compound to the superoxide anion (figure 6A). This, in turn, undergoes the action of superoxide dismutase (SOD), which dismutes O_2^- into H_2O_2 (figure 6A), which, although not a free radical, is still a molecule with high oxidizing power, also causing cell necrosis. From the formed H_2O_2, other ROS are derived. The hydroxyl radical (OH) is produced through the Fenton and Haber-Weis reactions; In the Fenton reaction (figure 6B), H_2O_2 reacts with iron II ions, in the presence of copper (Cu^+), generating the hydroxyl radical (OH^-) and other compounds; in the Haber-Weiss reaction, O_2^- reacts with H_2O_2 in the presence of iron III and Cu^{2+} generating OH^-, as shown in figure 6C. In figure 3 it is also possible to observe the formation of hypochlorous acid (OHCl), a potent microbicide, through the oxidation of chloride ions by the previously formed H2O2, in a reaction mediated by MPO (MOURA *et al*., 2017; DINÇ *et al*., 2018; USLU *et al*., 2018; KOCAMAN *et al*., 2019).

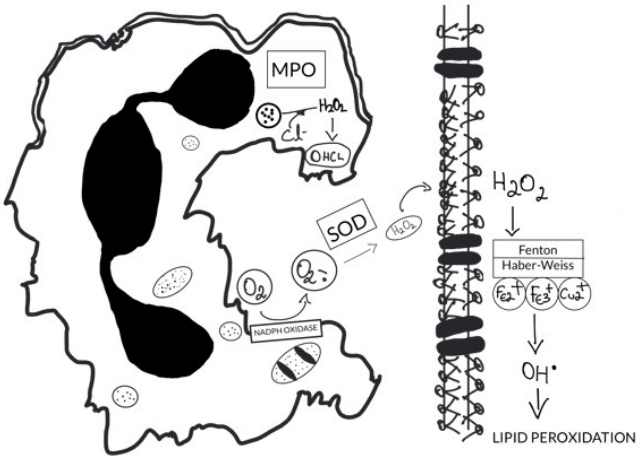


Figure 5. Illustration of oxidative stress from phagocytosis of antigens by neutrophils. The figure shows the activities of NOX, SOD and MPO enzymes

All these molecules described act mainly through lipid peroxidation, which consists of the attack carried out by ROS on phospholipids, causing rupture of the molecules that compose the microbiological membranes and the host cells; It is important to remember that any biomolecule can be attacked by ROS, such as proteins and nucleic acids (SCAREL-CAMINAGA *et al*., 2017).

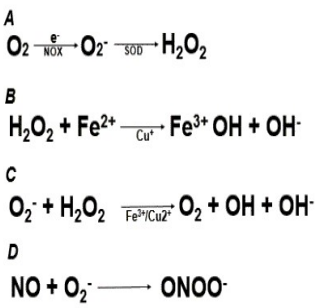


Figure 6. Main reactions involved in the production of ROS. A) Production of superoxide anion by the enzyme NADPH oxidase (NOX) and production of H_2O_2 by superoxide dismutase; B) Fenton reaction, H_2O_2 reacts with iron II in the presence of copper, forming iron III, hydroxyl and hydroxyl radical; C) Haber-Weiss reaction, the superoxide anion reacts with hydrogen peroxide in the presence of iron III and bivalent copper, forming molecular oxygen, hydroxyl and hydroxyl radical; D) Reaction between nitric oxide and superoxide anion, forming peroxynitrite. Reactions are not balanced.

Figure 6 contains a summary of the main ROS formation reactions. Furthermore, O_2^- can react with NO, producing peroxynitrite (figure 6D), a potent oxidizing and nitrating agent, related to a high rate of lipid peroxidation. Lipid peroxidation index is an important indicator of tissue loss in infectious and inflammatory diseases, and can be measured through the plasma levels of malondialdehyde (MD). It is important to remember that oxidative and nitrosative stresses are necessary for the immune defenses of the host, especially with regard to microbiological death, however, these compounds are also deleterious to host cells and their excesses are fought by the body's antioxidant systems, formed mainly by catalase, glutathione and thioredoxin systems (SLOSKY and VANDERAH, 2015).

CONCLUSION

Gingivitis is characterized as a condition of gum inflammation caused mainly by bacteria from the bacterial plaque in periodontal disease. When untreated, it evolves and causes attachment loss, accompanied by periodontal pocket formation and possible tooth loss. The recognition of plaque microorganisms causes the activation of phagocytes, which, in turn, initiate a process of inflammation through the production of inflammatory mediators, such as chemokines, which are responsible for chemotaxis, and cytokines, which activate recruited leukocytes. Phagocytosed microorganisms induce excessive production of ERNs and ROS, responsible for microbiological and gingival cell death. In addition, gingival tissue produces inflammatory mediators through the arachidonic acid cascade, initiated by phospholipase A, forming arachidonic acid, and the activity of COX enzymes, producing prostaglandins, prostacyclins, and thromboxanes; and LOX, producer of leukotrienes. Typical anti-inflammatory drugs such as SADs and NSADs, which block phospholipase A2 and COX enzymes, respectively, are the most common prescription drugs, with NSAIDs being the most used in gingivitis treatments, when necessary. Despite this topic being well discussed, several aspects of it still remain not fully elucidated, being highly debated and tested in the scientific community. Dentists are prescribers, therefore, understanding the mechanisms discussed here is extremely important for an effective prescription/assistance for the patient.

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