ORIGINALL ARTICLE

Role of Antimicrobial Peptides in Periodontitis

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ABSTRACT

Background: **Periodontitis is an infection-driven inflammatory disease in which the composition of biofilms plays a significant role. Dental plaque accumulation at the gingival margin initiates an inflammatory response that, in turn, causes microbial alterations and may lead to drastic consequences in the periodontium of susceptible individuals. Antimicrobial peptides protect the host against various pathogens such as bacteria, viruses, fungi and yeast. Antimicrobial peptides also display immunomodulatory properties ranging from the modulation of inflammatory responses to the promotion of wound healing.**

Aim of Study: **The purpose of this review is to outline the function of the antimicrobial peptides (cathelicidin, human neutrophil peptides 1-3 and human beta defensin-1) in periodontitis patients.**

Methodology: **Using suitable keywords, relevant papers would be searched in the scientific databases Scopus, Google Scholar, PubMed, and Web of Science.**

Results: **Covered studies were posted between 2000 and 2022 and detect great variance in patients selection, clinical assessments, and antimicrobial peptides measures. A significant association between antimicrobial peptides and periodontitis developments was reported in some studies.**

Conclusion: **antimicrobial peptides play a crucial role**

KEY WORDS

periodontal disease, antimicrobial peptides, definsin, LL-37, immune response

INTRODUCTION

Periodontitis is a chronic multifactorial inflammatory disease associated with the accumulation of dental plaque and characterized by progressive destruction of the teeth-supporting apparatus, including the periodontal ligament and alveolar bone[1)]. The disease involves complex dynamic interactions among specific bacterial pathogens, destructive host immune responses and environmental factors such as smoking. The common features of periodontitis include gingival inflammation, clinical attachment loss, radiographic evidence of alveolar bone loss, sites with deep probing depths, mobility, bleeding upon probing and pathologic migration[1,2)].

Antimicrobial peptides (AMPs) are a class of active oligopeptides that are toxic to pathogens[3)]. AMPs are widely distributed in nature, with examples reported from microorganisms, plants, invertebrates, fish, amphibians, birds and mammals[4,5)]. Because they mostly have a net positive charge and also exhibit hydrophobicity, AMPs can combine with negatively charged surface due to electrostatic interactions, penetrate and destroy the membrane structure, resulting in the death of bacteria, fungi, parasites and viruses[6)]. Different from the bactericidal principle of traditional antibiotics with a single target, AMPs can destroy pathogens by damaging multiple targets, which can greatly reduce the emergence of drug-resistant bacteria and makes them one of the best alternatives for comprehensive antibiotics due to their broad-spectrum antibacterial properties[4,5,7)]. There are two subfamilies of AMPs in mammals: cathelicidins and defensins. Both types of AMPs are part of the innate immune system. In humans, there are many classes of defensins, while there is only a single identified cathelicidin[8)].

Antimicrobial Peptides

Antimicrobial peptides, also called host defense peptides, exerting a cationic nature, are part of the innate immune response. AMPs are considered as endogenously produced antibiotics, and they act at an early stage against microbial invasion[9)]. Over 45 distinct AMPs have been identified in human saliva and GCF[10)]. They are produced by the salivary glands and epithelial cells, and they form a continuous layer on the mucosal surfaces[11)]. These AMPs have been reported to have distinct but overlapping roles in maintaining oral health and preventing bacterial, fungal and viral adherence and infection[12)]. Defensins, cathelicidins, calprotectins and histatins are the major AMPs detected in the oral cavity[13)]. AMPs participate in a preservative co-evolution with the microbiome, and they help to maintain a balanced microbiota. Furthermore, apart from their antimicrobial activity, the AMPs have been reported to participate in several other crucial roles in host tissues, such as wound healing and cell proliferation, and chemotactic for immune cells[14)].

Structures and Characteristics of Antimicrobial Peptides

Antimicrobial peptides are ubiquitous in nature. They exist in various organisms including bacteria, fungi, animals and plants, and in all other mammalian species[15)]. However, LL-37 consisting of 37 amino acids with two leucine residues at its N-terminus is the only one of the AMP family discovered in human[16)]. They usually contain a composition rich in cationic and hydrophobic amino acids, and have the cationic (positively charged) and amphiphilic (both hydrophilic and hydrophobic) characteristics, due to these AMPs containing the rich hydrophobic groups, and having both hydrophobic regions and hydrophilic

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regions[17,18]. These cationic AMPs generally are positively charged and helical polypeptides with short amino acid sequences (less than 100 amino acid residues) including excessive amounts of the positively charged amino acids lysine and arginine[19]. The amphiphilic peptide molecules are α-helices with hydrophobic and hydrophilic halves and display their amphiphilicity while interacting with bacterial cell membranes. These peptides fold into amphipathic α-helices with both hydrophilic and hydrophobic sides when adsorbed to the bilayer lipid membranes[17].

These positively charged AMPs interact with negatively charged cell membranes through electrostatic interactions and undergo membrane adsorption and conformational change. Peptides bind to the membrane surfaces with their hydrophobic sides anchored in the hydrophobic lipid core of the bilayer[20]. These peptides at their N-terminal ends are rich in basic amino acids with strong alkaline, and they at their C-terminal ends are amidated with C-terminal neutral hydrophobicity. The number of the cationic net charges of these peptides is related to the antibacterial activity, and their hydrophobicity is consistent with the hemolytic activity[21]. Some synthetic peptides can stabilize and keep biological activity at high temperature, and some can resist the hydrolysis of trypsin and pepsin[22]. AMPs are the important components of innate immunity. They can resist the invasion of foreign microorganisms and have broader spectrum antibacterial properties compared to the traditional antibiotics[19].

Role of Antimicrobial Peptides in Immune Responses

The cationic AMPs play important roles in the natural immunity of the hosts. They not only kill the pathogenic microorganisms that invade the human body, but also show their multiple functions at different stages of the natural immune responses[23]. These peptides can stimulate the proliferation of cells including fibroblasts, lymphocytes and vascular endothelial cells. They also promote the growth of wound granulation tissue and enhance wound healing[24]. These cationic AMPs are involved in the host defenses associated with acute inflammation. They can induce bacterial lysis, promote phagocytosis of macrophage, prevent infection spreading, stimulate mitosis of fibroblasts and epithelial cells, and promote fibroblast growth to enhance wound healing[24,25]. They can activate human lymphocytes to eliminate the cells infected with viruses and bacteria, and the cancer cells. These AMPs also play a role in chronic inflammation. They promote the proliferation of T helper cells and the production of chemokine in these T cells, increase the levels of antibody IgG inside body, promote apoptosis of macrophages, and activate lymphocytes to clear infected cells[23]. Moreover, a study reported by Ghufran *et al*. (2021)[26] support the idea that of a protective role of antimicrobial peptides as important immune molecules in maintaining the oral health against caries. Therefore, it could be a gate for more advanced future biological screening method for caries susceptibility and new strategies for prevention. On the other hand, other study showed that the reduction in b-defensin-1 levels indicates an abnormal immune response in these patients and may require a new treatment option for this condition in the future. Furthermore, in patients with severe disease, a considerable drop in b-defensin-1 might be used as an indicator of illness severity[27].

Mechanism of Action of Antimicrobial Peptides

Antimicrobial peptides interact with bacterial cell membrane through electrostatic interactions[28] thus making it difficult for bacteria to develop resistance unlike conventional antibiotics[29]. Based on their mode of action, these peptides are classified into membrane acting and non-membrane acting peptides. Membrane acting peptides mainly harbour cationic peptides causing membrane disruptions, whereas non membrane peptides are capable of translocation across the membrane without damaging it[30]. Few antibacterial peptides create trans-membrane pores on the target membrane and include defensin[31], melittin[32], againins[33], and LL-37[34].

Antimicrobial peptides such as buforin II[35], dermaseptin[36], HNP-1[37], pleurocidin[38], indolicidin[39], and mersacidin[40] these peptides translocates across the cell membrane and disrupt normal cell functioning[41]. Outer membrane of prokaryotic cell is negatively charged owing to presence of LPS or teichoic acid, whereas the outer leaflet of eukaryotic cell consists of zwitterionic phos-phatidylcholine and sphingomyelin phospholipids. Cationic AMPs interact with negatively charged outer microbial membranes via selective interactions[42], and attain well-define secondary structures, makes cell permeable and finally disrupt bacterial membranes[43]. These peptides show dynamics in structure and topologies during their interactions with the microbial cell membranes[44]. AMPs also hamper processes like protein synthesis, nucleic acid synthesis, enzymatic activities and cell wall synthesis[45]. Several factors that include magnitude and charge of the outer membrane, concentration of negatively charged molecules, molecular architecture, and membrane fluidity are essential for the transportation of peptide across the membrane[46].

The membrane fluidity also regulates adsorption and insertion of AMPs into the cell membrane. Malanovic and Lohnerin in (2016)[47] studied AMPs against Gram positive bacteria and found that prior targeting the cytoplasmic membrane these peptides cross the cell wall components such as lipoteichoic acids and peptidoglycan. It was established that highly conserved precursors of cell wall components, especially lipid II are directly targeted by AMPs[48]. Majority of these AMPs fold into amphipathic conformations while interacting with membrane[49]. Some of the AMPs crosses lipid bilayer, target intracellular components, binds DNA, block enzyme activity, inhibit synthesis of proteins, cell wall, and nucleic acids[37].

Immunoregulatory Functions of Antimicrobial Peptides

It has been demonstrated that AMPs perform a broad range of immunomodulatory functions beyond their antimicrobial activity[50]. The molecular mechanism by which AMPs regulate immune responses is highly complex, and immunomodulatory activity varies depending on the environmental stimuli, cell type and peptide concentration[51]. Immunomodulatory activities of AMPs include stimulating chemotaxis of immune cells, modulating neutrophil function, and influencing adaptive immunity by recruiting antigen presenting cells (APCs) to infection sites. AMPs function as chemoattractants to stimulate chemotaxis of leukocytes by secreting chemokines[52].

In addition, AMPs regulate neutrophil functions by stimulating the release of neutrophil chemokines or increasing neutrophil influx through chemotactic functions[53]. Antimicrobial peptides are also present in neutrophil extracellular traps (NETs) and are involved in NET-mediated antibacterial effects[54]. Upon infection, AMPs recruit APCs, such as monocytes, macrophages and dendritic cells, and mediate innate and adaptive immunity. AMPs exert both pro- and anti-inflammatory properties depending on the cell type and inflammatory stimuli, thereby establishing a balance of inflammation. The anti-inflammatory functions of AMPs are highlighted in studies on the association between low α-defensin expression and ileal Crohn's disease, and in a report that a cathelicidin-knockout mouse model showed more severe inflammatory responses than the wild type[55]. On the other hand, cathelicidin can also promote inflammation through the induction of proinflammatory cytokines and chemokines or DNA- and RNA-mediated TLR activation[56]. Thus, overproduction of AMPs can directly trigger inflammatory diseases such as psoriasis, which highly express AMPs such as cathelicidin, β-defensins, and S100 proteins in their lesions[57].

Human Neutrophil Peptides

The mature human oral α-defensins peptides are small, single, arginine-rich amino acids in sequence with 30-50 amino acid residues conserved with six cysteine motifs. They are synthesized as pre and pro-peptides, expressed from neutrophil hence, called as HNP[58]. HNP-1, -2, -3 and -4 are stored as mature peptide in granules of neutrophils and human defensins, α-defensins (HNP-5 and HNP-6) are released as a pro-peptide extracellularly[59]. HNP 1-3 have similar amino acids sequence only first residue of HNP-1 starts with alanine and HNP-3 with aspartate in their N terminus. The killing of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* was high in HNP-1 and HNP-2 in comparison to HNP-3[60]. The other family members of defensins family HNP-5 and HNP-6 were expressed in intestinal paneth cells and with no evidence of their presence in oral cavity. HNP -1, HNP-2 and HNP-3 showed inhibitory effect against *human immune virus*[61], *Herpes Simplex Virus* (HSV)-1, HSV-2, *vesicular stomatitis virus*, *influenza virus*, *cytomegalovirus*, *papillomavirus* and *adenovirus*[62]. Human neutrophil peptides 1-3 are the most abundant forms and are stored in the azurophilic granules of neutrophils. It was reported that plasma and other body fluids contain elevated concentrations of HNP1-3 during infection[63]. Puklo *et al*. (2008)[64] reported that HNP1-3 was elevated in periodontitis patients compared to control. Similarly, Mizukawa *et al*. (1999)[65] showed that the concentration of HNP 1 was higher in the saliva of patients with oral inflammation than in healthy subjects. In addition, McKay *et al*. (1999)[66] suggested that high levels of HNP1-3 in GCF indicate the effects of these AMPs in controlling of the microbiota of the gingival crevice. The possible explanation for the high level of HNP1-3 in periodontitis could be attributed to that sources other than

neutrophils are responsible for α-defensin production in inflamed periodontal/gingival tissue. Indeed, it has been shown that HNP1-3 is also expressed in lymphocytes and monocytes, which are abundant in periodontitis sites[67]. On the other hand, Lundy *et al*. (2005)[68] examined the α-defensins (HNP1-3) in periodontitis and periodontally healthy and reported that there was no significant difference between healthy and diseased sites although these defensins were more abundant in a higher proportion of the healthy sites.

Cathelicidins (LL-37)

Cathelicidins (LL-37) are AMPs from the family of a-helical peptides without cysteine and located at the carboxyl terminus of a 15-18 kDa highly conserved cathepsin-L-inhibitor (cathelin)-like domain[16]. Cathelicidins only have one delegate in humans in the oral cavity and respiratory tract which is known as human cationic antimicrobial peptide (hCAP18)[69]. They are synthesized and stored in cells as 2-domain proteins and when required are split by proteases to produce a cathelin protein and an AMP. They derive their name from the first two residues at the N-terminus (Leucine, Leucine) and contain 37 amino acids[70]. Leucine leucine-37/human cationic antimicrobial peptide-18 has the function of stimulation of monocytes, neutrophils, mast cells and T-cells. Various studies have demonstrated the capability of LL-37/hCAP18 as a potent antimicrobial against many Gram-negative and positive bacteria, fungi, viruses and parasites[11]. LL-37/hCAP18 neutralizes bacteria very quickly by forming ionic channels in the cell membranes of the microorganisms and by ability to bind LPS of bacterial membranes[70]. A study demonstrated the stronger killing action of LL-37/hCAP18 derived synthetic peptides against *Streptococcus* (*S.*) *sanguinis* isolated from Behcet's disease. In addition, Ouhara *et al*. chemically synthesized HBD-1, HBD2, HBD3 and LL-37 (CAP18) for their antimicrobial activity against oral bacteria (*S. mutans*, *S. sanguinis*, *S. salivarius* and *S. mitis*) and demonstrated the high activity of LL-37 against these pathogens[11,12]. Neutrophils and neutrophil-derived AMPs play a key role in maintaining the balance between health and disease. LL-37 is an important AMP in defending periodontal tissues from microbial pathogens in the oral cavity[64]. Cheah and colleagues showed that serum LL-37 level was significantly higher in periodontitis group than periodontal health group and indicated that subjects with high serum LL-37 should receive comprehensive periodontal therapy[71]. Moreover, Puklo *et al*. (2008)[64] have demonstrated that unprocessed LL-37 levels in patients with periodontitis were significantly higher compared to the healthy control. An increase of this AMP may be due to the association of LL-37 with inflammation during periodontal disease. However, Eick *et al*. (2014)[72] failed to show any differences in the level of LL-37 between periodontitis patients and healthy control.

In contrast, Turkoglu and colleagues (2017)[73] observed that patients with periodontitis had significantly low levels of serum LL-37 than healthy control. This conflicting result might be caused by different techniques of the studies, ELISA technique might detect not only the mature form but also immature forms of hCAP18/LL-37. This peptide is stored in secondary granules of neutrophils as an inactive precursor[74]. After the stimulation of neutrophils, mature hCAP18/LL-37 is released from hCAP18 by proteinase 3[75]. Recruited neutrophils releasing hCAP18/LL-37 peptide act an important role in non-oxidative killing mechanism[76]. It has been shown that mature hCAP18/LL-37 has a killing effect on *Aggregatibacter actinomycetemcomitans*[77], which is an important periodontopathogen for periodontitis[78]. Because LL-37 possesses a broad spectrum of antimicrobial properties and can directly kill a variety of Gram-positive and Gram-negative bacteria[79], it is likely to have a role in immune defense processes during infectious disorders.

Beta-Defensins

First isolation of β-defensins peptide is done in 1993 from bovine tracheal cells with a range of 38-42 amino acids peptides residue. In protein data bank six-human beta defensins, HBD-1, HBD2, HBD-3, HBD-4, HBD-5 and HBD-6 were identified respectively, whereas, six gene-based β-defensins HBD-25, HBD-26, HBD-27, HBD-28, HBD-29, and HBD-31 were reported. They are constitutively expressed in the different epithelial surface of the human body. HBD-1, HBD-2 and HBD-3 were isolated from human plasma and oral tissues which include gingiva, parotid gland, buccal mucosa and epithelial lining of the tongue, respiratory tract, pancreas, kidney and uterus[80]. Human beta defensin-3 were also expressed from the placenta, adult heart muscles, skeletal muscle, fetal thymus, oesophagus and trachea[81]. HBD-4 has a classic structure, it comprises of two exons, one encodes signal peptides, and another exon encodes the pro-peptide which has been expressed in thyroid, lungs, kidneys, stomach, cells of the testis, and uterus. HBD-5 and HBD-6 are explicitly expressed in epididymis cells. The HBDs families have a substantial role in defense of mucosal and epithelial surfaces[82]. Human beta defensin-1 controls the healthy interaction between the microbiota and epithelial surfaces in health and takes part in innate immune defense in disease[83]. Yilmaz *et al*. (2020)[84] reported that patients with periodontitis had higher levels of salivary HBD-1 and HBD-3 in comparison to periodontally healthy control. Similarly, Lu *et al*. (2004)[85] found that HBD-1 mRNA levels in the pocket epithelium of patients with severe periodontitis were higher than those in the healthy sulcus epithelium of the same patients. The presence of pathogenic microorganisms and the presence of an inflammatory process could both cause HBD-1 levels to rise. Cytokines attract plasma cells to the epithelium during bacterial infection and subsequent inflammation, allowing HBD-1 to be released into the tissues[86]. According to Mathews et al. (1999)[87] continuous contact of the oral mucosa with the commensal microflora can stimulate HBD-1 production even in the absence of periodontal disease.

On the contrary, Costa and colleagues (2018)[88] reported that periodontally healthy individuals had significantly higher levels of HBD-1 when compared to individuals with periodontitis and suggested a potential protective role of HBD-1 in periodontitis susceptibility. Dommisch *et al*. (2005)[89] found decreased human HBD-1 mRNA levels in the gingiva of patients with periodontitis compared with healthy control; however, the difference was not statistically significant. This disparity in results could be due to differences in the number of gene copies and responses to bacterial stimulation. Aside from methodological variations, the sample age and stage of periodontitis in the studies are other factors. However, recent study reported that Vitamin D deficiency significantly associated with periodontitis. The assessment of Vitamin D levels in patients with periodontal disease seems advisable, as Vitamin D deficiency might be involved in the disease onset and progression. Moreover, low levels of LL-37, and HBD-1 in periodontitis patients with insufficient Vitamin D, implying that vitamin D plays a role in AMPs production[90].

MOREVER CONCLUSIONS

High levels of cathelicidin, human neutrophil peptides 1-3 and human beta defensin-1 in patients may form the initial response against infection and thus could function as an early diagnostic marker of periodontitis.

CONFLICTS OF INTEREST

Authors declare that there is no conflict of interest.

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