



Science

HOST PROTEASE INHIBITION BY SPECIFIC PATHOGENS IN PERIODONTAL DISEASE

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Abstract

Proteolytic tissue degradation is a typical phenomenon in chronic inflammatory periodontal disease with the uncontrolled release of host and bacterial derived proteases causing self-digestion and tissue destruction. Antimicrobial proteins and peptides constitute a diverse class of host defense molecules that act early to combat invasion and infection with bacteria and other microorganisms and protease inhibitors forms one of the functional classes of antimicrobial peptides. Plasma protease inhibitors present in gingival crevicular fluid as well as tissues may play a critical role in the protection of periodontal tissues by modulating protease activity, more particularly during active phases. This literature review attempts to highlight the role of host protease inhibitors and their interaction with specific periodontal pathogens in the pathogenesis of periodontal disease.

Keywords: Proteases; Antimicrobial Peptides; Protease Inhibitors; Gingipains.

Cite This Article: Saipriya S, and Vijay Kumar Chava. (2018). “HOST PROTEASE INHIBITION BY SPECIFIC PATHOGENS IN PERIODONTAL DISEASE.” *International Journal of Research - Granthaalayah*, 6(4), 131-137. <https://doi.org/10.5281/zenodo.1242613>.

1. Introduction

Proteolysis is an important phenomenon, contributing to the progression of chronic inflammatory periodontal disease. Proteases and protease inhibitors, an early component of innate responses mediates the host microbial interactions. The proteolytic enzymes can be derived from both the host and bacteria. The uncontrolled release of these enzymes in inflammation results in self digestion and tissue destruction. On the other hand, the host protease inhibitors (antimicrobial peptides) found in oral fluids combat invasion and infection with microorganisms. The bacterial derived protease confers its effect either directly with their collagenolytic action or indirectly by digesting these host protease inhibitors.¹

The expanding horizon of knowledge on the mechanism and various interactions leading to chronic inflammatory periodontal disease has thrown light on the importance of the balance of proteases and their inhibitors in the extent and progression of the disease process. Emphasis is laid on application of these aspects in regulating the interactions with newer pharmacological agents and other innovations.

2. Host Proteolytic System

Host proteolytic system plays a crucial role in controlling periodontal tissue turnover in health and in tissue destruction that characterizes diseases of the periodontium. It can be categorized as

2.1. Plasma Proteases

Present as inactive zymogens in the blood and activated in inflammation. It includes the complement system, kallikrein kinin system and fibrinolytic system. Complement cascade generates peptide fragments that functions as anaphylatoxins (C3a and C5a), chemotactic factors (C5a and C5-C6-C7) and opsonins (C3b, C5b). Kallikrein kinin system when activated produces bradykinin a nonapeptide, increases vascular permeability by vasodilation, contracts smooth muscle, produces pain. Kallikrein is chemotactic to and promotes aggregation of neutrophils. Fibrinolytic clotting system is the third protease cascade that results in the formation of fibrin. ²

2.2. Neutrophil Proteases

Are the heterogeneous molecules stored in the neutrophils ³ used for host defense as shown in Table 1

Table 1: Neutrophil Proteases

PROTEINASE	SUBSTRATE
Leukocyte elastase	Elastin, collagen III,IV,VI,VIII, Fibronectin, laminin1, Thrombospondin, proteoglycans
Cathepsin G	Fibronectin, laminin1, proteoglycan, Collagen IV, elastin, immunoglobulin
Proteinase3	Fibronectin, laminin1, vitronectin, Proteoglycan, collagen IV, elastin
Plasminogen activator μ PA	Plasminogen (plasmin degrades fibrin, fibronectin, laminin1, Thrombospondin, proteoglycans & activates pro-mmp & complement
Leukocyte collagenase (MMP-8)	Collagen I,II,III,VII,X, bradykinin, Angiotensin I, substance P, α 1-proteinase inhibitor, tenasin, entactin, aggrecan, α 2-macroglobulin
Gelatinase (MMP-9)	Collagen IV,V,VII,X,XI, gelatin, Fibronectin, vitronectin, laminin, elastin, aggrecan
Cathepsin S,L,B,H	Elastin, denatured proteins, activation of pro-MMPs, pro-urokinase
Cathepsin D,E	Proteoglycans, denatured proteins

2.3. Epithelial Cell Proteases

The relatively passive epithelial cells when activated, can behave very aggressively by migrating, proliferating, and producing various cytokines and proteolytic enzymes. Therefore they play an active role in inflammation and in defense against microbes.

2.4. Fibroblast Proteases

Fibroblasts responsible for the turnover of connective tissue have the capacity to degrade its components including different collagen types, adhesion proteins and proteoglycans. The major MMPs produced by fibroblasts are MMP-1 (collagenase-1), MMP-13 (collagenase-13), MMP-2 (gelatinase A), MMP-3 (stromelysin-1), MT1-MMP (MMP-14), MMP-8, Cathepsins B and L and Tissue plasminogen activator.

3. Proteolytic System of Specific Periodontal Pathogens

The microorganisms utilize the peptides in the periodontal pocket, as potential carbon and energy sources for their growth by the joint action of oligopeptidases and exopeptidases ⁴ as described in Table 2. The various effects of proteases of specific pathogens ⁵ causing alterations in the host proteins in favor of the pathogenesis of periodontal disease are depicted in Figure 1

Table 2: Proteases of Major Periodontopathogens

PORPHYROMONAS GINGIVALIS	Gingipains R
	Gingipain K
	Periodontain
	PrT proteinase
	Tpr proteinase
	Collagenase
	Prolyl tripeptidyl-peptidase (PtpA)
	Dipeptidyl-peptidase IV (DPP IV)
	Dipeptidyl-peptidase VI
	Amino-peptidase P
	Oligo-peptidase O
	True collagenase
	Gelatinase (collagenase, Pz-peptidase)
TREPONEMA DENTICOLA	Trepolisin
	Proline aminopeptidase
	Prolyl oligopeptidase
	Endopeptidase
	Arginine specific oligopeptidase
	Chymotrypsin like protease
TANNERELLA FORSYTHIA	Trypsin-like protease
	prtH protease
A. ACTINOMYCETEMCOMITANS	Trypsin like protease

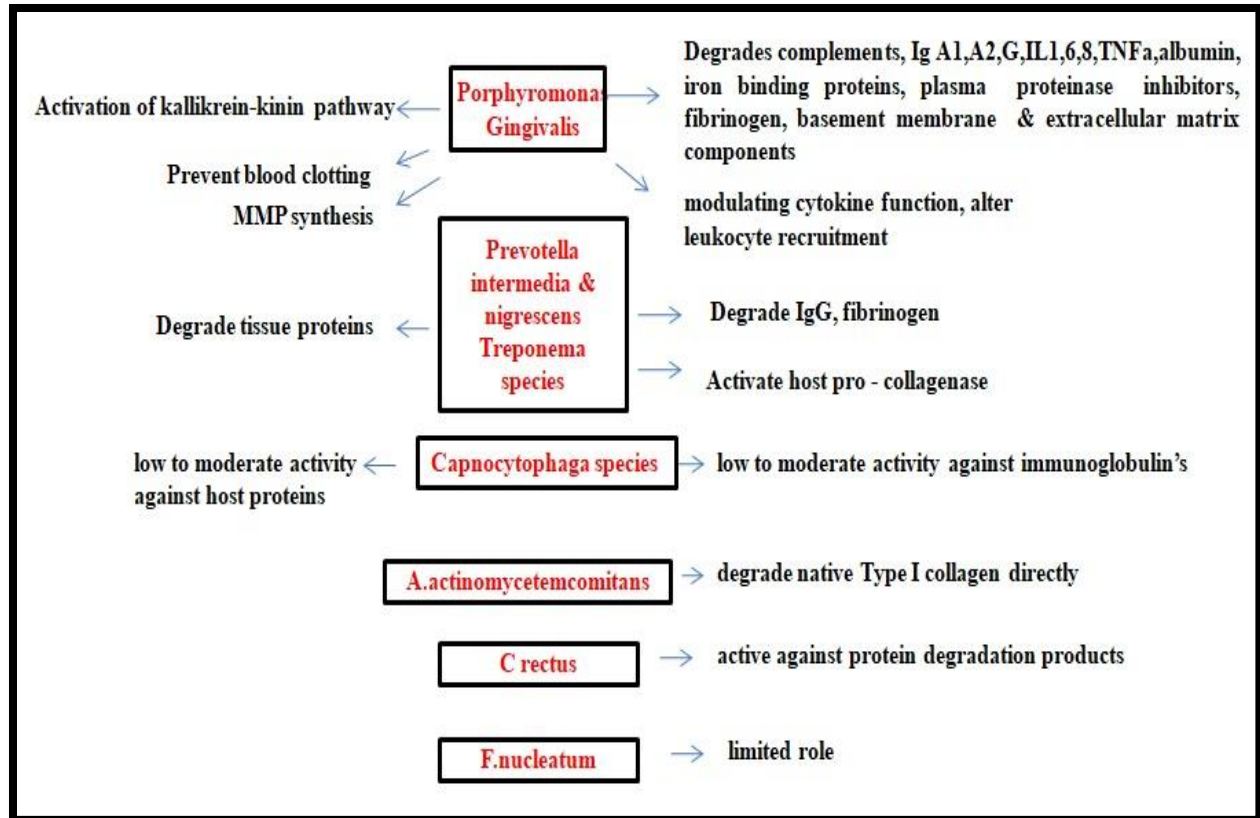


Figure 1: Effects of Specific Pathogens on Host Proteins

4. Host Protease Inhibition

Antimicrobial peptide: One of the six functional families of antimicrobial peptides affecting the course of periodontal disease by inactivating bacterial or host proteases or binding bacterial toxins are *protease inhibitors*. This includes Cystatin A, Cystatin B, Cystatin C, Cystatin D, Cystatin S, Cystatin SA Cystatin SN, Secretory leukoprotease inhibitor protein and Skin derived antileukoprotease (SKALP)/Elafin (ESI-elastase-specific inhibitor).⁶

Plasma protease inhibitors: The activity of proteolytic enzymes in the tissues is modulated by plasma derived and locally produced inhibitors. One of the major functions of the plasma proteinase inhibitors is to modulate the activity of proteinases released by the polymorphonuclear leukocytes involved in the host defense against the microbes. The main inhibitors of the leukocyte proteinases are alpha-1-antitrypsin, alpha-1-antichymotrypsin, and alpha-2-macroglobulin.⁷

5. Action of Protease Inhibitors and Specific Pathogens

The role of protease inhibitors is to create a balance and maintain health of the tissues, whereas the periodontal pathogens with its armamentarium of proteases take part not only in tissue destruction but also in inactivating these protease inhibitors as described in Table 3 & 4.

Table 3: Action of Protease Inhibitors

Secretory leukoprotease inhibitor (SLPI) potent inhibitor of serine proteases, including neutrophil elastase (NE), cathepsin G, mast cell chymase and a chymotrypsin-like enzyme	protects the host during bacterial infection, antagonizing bacterial toxins such as LPS, suppressing matrix metalloproteinase production and activity and inducing the anti-inflammatory cytokine interleukin (IL)-10
Skin-derived antileukoproteinase (SKALP)/Elafin potent inhibitor of the neutrophil serine proteases elastase and proteinase3.	The protein kills both gram-negative and gram-positive bacteria; this activity depends on the presence of both peptide domains (Simpson et al 1999)
Cystatins cysteine protease inhibitors that block the action of bacterial proteases on target tissues	Cystatins C and S inhibit the growth of P. gingivalis.(Baron et al 1999)
Alpha-2-macroglobulin	principal regulators of both endogenous and exogenous proteases
Alpha-1-antitrypsin	specific for serine proteinases
Alpha-1-antichymotrypsin	potent inhibitor of cathepsin G

Table 4: Action of Specific Pathogens on Protease Inhibitors

Into et al 2006, Lei Yin et al 2010	gingipains might be responsible for reducing the level of SLPI by cleaving its domains
Kantyka et al 2009	all three gingipains (RgpA, RgpB, and Kgp) were found to degrade elafin, with RgpB being the most efficient gingipain
Grenier et al 1996	trypsin-like protease (80 kDa) from P. gingivalis and a chymotrypsin-like protease (95 kDa) from T. denticola could degrade and inactivate cystatin C
Abrahamson et al 1997	P gingivalis and P intermedia have the capacity to modify cystatin proteolytically
Elkaim et al 2008	P.gingivalis in human oral epithelial cells creates an imbalance between Cathepsin B and the inhibitor Cystatin C
Carlson et al 1984, Fishburn et al 1991, Herrmann et al 1985 Nilsson et al. 1985, Uitto et al 1988	Trypsin-like protease (80 kDa) from P. gingivalis and a chymotrypsin-like protease (95 kDa) from T. denticola, degrades most serum proteins including the plasma proteinase inhibitors alpha-1-antitrypsin, alpha-2-macroglobulin, antichymotrypsin, antithrombin III

6. Discussion

In many respects pathogenesis of periodontal diseases results from an interaction of certain periodontal pathogens with host immune responses. According to Garant 2003⁸, a primary host-response to bacteria colonizing the sub gingival tooth surface is infiltration of the gingival tissue

and sulcus by large numbers of neutrophils (PMNs) which constitute the main source of proteolytic activity and antimicrobial peptides.

The development of periodontal diseases is associated with an increased level of proteolytic activity in sub gingival sites, including the gingival crevicular fluid (Eley & Cox, 1992⁹ Sandholm 1986¹⁰). These enzymes, which originate from both the host and periodontopathogens, participate in the pathogenesis of periodontal diseases through their action on host proteins.

Latest Updates on Synthetic Antimicrobial Peptides

A novel synthetic cationic antimicrobial peptide, **Nal-P-113**¹¹, which inhibits and kills periodontal bacteria in planktonic state, inhibits the formation of biofilms and eradicates polymicrobial biofilms. Nal-P-113 is also stable in saliva, serum and saline solution. At a concentration less than 320 µg/mL which is harmless to normal oral cells, Nal-P-113 can kill bacteria in planktonic state.

A peptide that contained three cationic amino acids (Arg, His and Lys), two anionic amino acids (Glu and Asp), hydrophobic amino acids residues (Leu, Ile, Val, Ala and Pro) and hydrophilic residues (Ser and Gly) was obtained and named **Pep-7**¹². The Pep-7 inhibited two pathogenic *P. gingivalis* ATCC 33277 and *P. gingivalis* ATCC 53978 (wp50) strains at a minimum bactericidal concentration (MBC) of 1.7 µM, but was ineffective against other oral microorganisms (*P. intermedia*, *Tannerella forsythensis*, *Streptococcus salivarius* and *Streptococcus sanguinis*). From transmission electron microscopy studies, Pep-7 caused pore formation at the poles of the cytoplasmic membranes of *P. gingivalis*.

Theaflavins¹³, the main polyphenols in black tea, possesses a wide range of beneficial pharmacological properties. Theaflavins exhibited the antimicrobial effects against both planktonic culture and biofilm of *P. gingivalis*. Theaflavins also markedly inhibited the proteinase activities of *P. gingivalis* collagenase and gingipains in a dose-dependent manner. Lastly, theaflavins significantly inhibited the secretion and mRNA expression of MMP-1 and MMP-2 by HGFs stimulated with *P. gingivalis*. Theaflavins can affect the virulent properties of *P. gingivalis* and attenuate the MMP-mediated inflammatory response induced by this pathogen, which suggests that theaflavins may be potentially valuable supplementary therapeutic agent for prevention and treatment of *P. gingivalis* associated periodontal diseases.

7. Conclusion

The virulence factors contribute to the production of disease in the host by acting as toxins or agents that overstimulate host immune defenses. One of the important classes of virulence factors are the proteases, which function in invasion, immune evasion, nutrition, and reproduction of the pathogen. Unsurprisingly, the host produces inhibitors that are capable of inactivating and clearing the proteases involved in pathogenic invasion.

Thus this knowledge regarding host microbial interactions has unfolded various therapeutic options to control the disease process and awaits further innovations.

Conflict of Interest

There is no conflict of interest

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