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Saudi Pharmaceutical Journal

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REVIEW

Histatin peptides: Pharmacological functions and their applications in dentistry

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Received 19 October 2015; accepted 26 April 2016

KEYWORDS

Saliva;
Oral cavity;
Histatin;
Antifungal activity

Abstract There are many human oral antimicrobial peptides responsible for playing important roles including maintenance, repairing of oral tissues (hard or soft) and defense against oral microbes. In this review we have highlighted the biochemistry, physiology and proteomics of human oral histatin peptides, secreted from parotid and submandibular salivary glands in human. The significance of these peptides includes capability for ionic binding that can kill fungal *Candida albicans*. They have histidine rich amino acid sequences (7–12 family members; corresponding to residues 12–24, 13–24, 12–25, 13–25, 5–11, and 5–12, respectively) for Histatin-3. However, Histatin-3 can be synthesized proteolytically from histatin 5 or 6. Due to their fungicidal response and high biocompatibility (little or no toxicity), these peptides can be considered as therapeutic agents with most probable applications for example, artificial saliva for denture wearers and salivary gland dysfunction conditions. The objectives of current article are to explore the human histatin peptides for its types, chemical and biological aspects. In addition, the potential for therapeutic bio-dental applications has been elaborated.

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Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

<http://dx.doi.org/10.1016/j.jpsps.2016.04.027>

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Please cite this article in press as: Khurshid, Z. et al., Histatin peptides: Pharmacological functions and their applications in dentistry. Saudi Pharmaceutical Journal (2016), <http://dx.doi.org/10.1016/j.jpsps.2016.04.027>

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1. Introduction

The significance and beneficial roles of organic macromolecules isolated from human body secretions are well known. Human peptides are secreted physiologically by certain organs such as salivary glands (Trindade et al., 2015; Kotál et al., 2015) or by pathologically by diseased tissues such as ameloblastoma (Jhamb and Kramer, 2014; Vered et al., 2003; Garg et al., 2015). In last few decades, a number of major developments such as better understanding and advent of proteomic tools started helping in isolation of protein based material from human secretions for example lysozyme (Sabatini et al., 1990), α -defensin, β -defensin, cathelicidins (Selsted et al., 1985), histatin family (Oppenheim et al., 1988), statherin, granulysin, thrombocidin-1, chemokine CCL20, psoriasin S100A7, neuropeptide (Dawidson et al., 1996), substance P and dermcidin (Zasloff, 2002). Reported secretions that have been mainly utilized included blood, lymph, saliva, glands secretion (endocrine and exocrine glands), urine, and body fluids (Schrader and Schulz-Knappe, 2001; Hu et al., 2006). Proteomics is a discovery science technology that helped categorizing genomics sequencing, physiology, microarray analysis and metabolite profiling of proteins and peptides (Cellulaire, 2002). Commonly used proteomic instruments are two-dimensional (2D) gel electrophoresis, liquid chromatography (LC), mass spectrometry with electrospray ionization (ESI), and matrix-assisted laser desorption ionization (MALDI) coupled with time of flight (TOF) (Gorg et al., 2004; Wittmann-Liebold et al., 2006).

These techniques and instruments can be used for the analysis of body fluids. For example, in case of oral and dental health, human saliva can be analyzed to differentiate between diseased and healthy patients without any surgical interventions (De Smet and Contreras, 2005). Saliva contains a vast number of protein species. The salivary peptidome (low molecular weight) comprises approximately 40–50% of the total secreted proteins in addition to peptides generated by proteolysis of proteins from different sources (Vitorino et al., 2004; Hu et al., 2005; Loo et al., 2010; Thomadaki et al., 2011). The timeline of human salivary proteins and peptides identification is listed in Table 1 (Hu et al., 2005; Piludu et al., 2006; Wong, 2006; Rudney et al., 2009). Up till now, more than 1165 types of salivary proteins have been identified and reported in human salivary proteome central depository (http://www.skbucla.edu/cgi-bin/hspmscgi-bin/welcome_c.cgi) supported by National Institute of Dental and Craniofacial Research (NIDCR). The objectives of current article are to explore the human histatin peptides for its types, chemical and biological aspects. In addition, the potential for therapeutic bio-dental applications has been elaborated.

2. Histatin peptides

Histatin peptides belong to a family of antimicrobial peptides that are rich in histidine amino acids. The first ever histatin was isolated from human parotid salivary gland secretions in 1988 (Oppenheim et al., 1988). Histidine rich polypeptides have been proven to have antimicrobial and antifungal properties (VAN et al., 1997). They are secreted by major salivary glands including parotid and submandibular glands. The concentration of histatin peptides in saliva ranges from 50 to 425 $\mu\text{g/ml}$ (VAN et al., 1997). Based on chemistry and sequence of amino acids, there are variety of histatin peptides. The common variants of natural histatins found in saliva are Histatin-1 (38 amino acids; Mw \sim 4929 Da), histatin-3 (32 amino acids; Mw \sim 4063 Da) and Histatin-5 (24 amino acids; Mw \sim 3037 Da) (Sabatini and Azen, 1989; Raj et al., 1990; Troxler et al., 1990). Histatin-1 and Histatin-3 are derived from the available genes HTN1 and HTN3 present in humans (Table 2) (VAN et al., 1997). Histatin-5 is originated from parent peptide i.e. histatin-3 and contains N-terminal that is thought to be highly reactive and highly affinitive to bond with metals. The chemical nature leads to precipitate reactive oxygen species (Nikawa et al., 2002; Cabras et al., 2007).

The analysis of Amino acid sequence of 12 histatin peptides (Table 3) suggested that histatin-2 is primarily degradable product of histatin-1. On the other hand, remaining histatins are proteolytic product of histatin-3. Considering the fact that histatins are humans own defense peptides, these peptides have gained popularity in the field of therapeutic and biodental medicine. In addition, the antimicrobial drugs containing natural peptides prevent resistance development against pathogens such as bacteria, fungi, viruses and parasites (De Smet and Contreras, 2005; Ryley, 2001; Wang, 2014).

3. Role of histatin peptides in human oral cavity

We are very well aware of the protective role of saliva that aids in digestion, lubrication, protection, and host defense immunization of the oral cavity (Dawes et al., 2015). These processes are made possible due to its unique composition and chemistry. Human saliva bio-fluid instead of blood can be used as disease biomarkers for diagnosis and therapeutic targets. In addition, it has major benefits of being cost effective and non-invasive not causing any pain or discomforts (Thomadaki et al., 2011; Halgand et al., 2012). Human saliva contains various forms of anti-microbial polypeptides that play few vital roles such as innate immunity and combating against invading foreign pathogens (Lamkin and Oppenheim, 1993), wound healing (Oudhoff et al., 2008), and apoptosis (Rudney et al., 2009). Among various peptides, histatin has

Table 1 Timeline of human salivary protein (<http://aps.unmc.edu/AP/>).

Proteins and peptides	Year	Amino acids	Functions
Statherin	1977	43	Potent inhibitor of calcium phosphate precipitation
α -Defensins (HNP-1-4)	1985	< 50	Antibacterial, antifungal and antiviral activities because of six cysteine disulfide bonds
Histatin-1	1988	38	Responsible for maintenance of oral hemostasis, role in forming acquired tooth pellicle and help in bonding of some metal ions
Histatin-3	1988	32	Responsible for maintenance of oral hemostasis, role in forming acquired tooth pellicle and help in bonding of some metal ions
Histatin-5	1988	24	Responsible for maintenance of oral hemostasis, role in forming acquired tooth pellicle and help in bonding of some metal ions
Adrenomedullin	1993	52	Mitogenic, inducible, vasodilator and antibacterial peptide
β -Defensins (hBD-1)	1995	36–47	Poor antibacterial activity, antiviral and antifungal activity, Also help in tissue repair
Cathelicidins (LL-37)	1995	37	Chemo-attractant to immune cells and act as “alarmin” by inducing immune response
β -Defensins (hBD-2)	1997	41	Antibacterial against Gram-negative and Gram-positive, antiviral and antifungal activity against HIV
β -Defensins (hBD-3)	2001	45	Antibacterial against Gram-negative and Gram-positive, also drug resistant microbes, chemo-attractant antiviral and antifungal activity
C-C motif Chemokine 28	2008	128	Act as chemokine, salt sensitive and broad spectral antibacterial
Azurocidin		251	Strong antibacterial activity against Gram-negative bacteria
Neuropeptides	1997	–	They have five types; Substance P (SP), Neurokinin A (NKA), Calcitonin Gene Related Peptide (CGRP), Neuropeptide Y (NPY) and Vasoactive Intestinal Polypeptide (VIP), effective against <i>Candida albicans</i> and bacteria
Calprotectin	1999	93–114	Act as calcium sensors and potent zinc binder, also play role in tissue repair
Mucins	1990	3750	Aggregate oral flora bacteria and prevent dental caries

Table 2 Representing the gene encoding series of human histatin family.

Allele	Gene	Protein
HIS1	HTN1	Histatin-1
HIS2	HTN3	Histatin-3 – histatin-4 to -6

been targeted and gained quite interests in field of biodental research utilizing proteomics approach (Khurshid et al., 2016; Siqueira et al., 2012). The eminent forms of human histatins are Histatin-1, Histatin-3 and Histatin-5 with individual contribution of around 20–30% to the total histatin pool. The theorized functions of histatin are buffering (Amado, 2005), wound healing (Kavanagh and Dowd, 2004), anti-candidal (Tsai and Bobek, 1998), antiviral (Nikawa et al., 2002), antibacterial (Cabras et al., 2007; Ryley, 2001) and balance of minerals formation (Wang, 2014).

In 2011 McDonald et al. worked on Histatin-1 and studied the composition, structure and function of acquired enamel pellicle. The key role of Histatin-1 absorbed by hydroxyapatite crystals was to protect from proteolytic degradation and demineralization (McDonald et al., 2011). Histatin 1 comprises phosphorylated serine at number 2 residue that increases affinity of the peptide to bind with HA crystals (Yin et al., 2003). One of the distinctive features of human oral mucosa is that, it has a high turnover rate. Hence, this characteristic is considered due to the presence of many factors such as presence of Histatin-1 and Histatin-2. These peptides indirectly induced wound healing by stimulating epithelial migration (Oudhoff

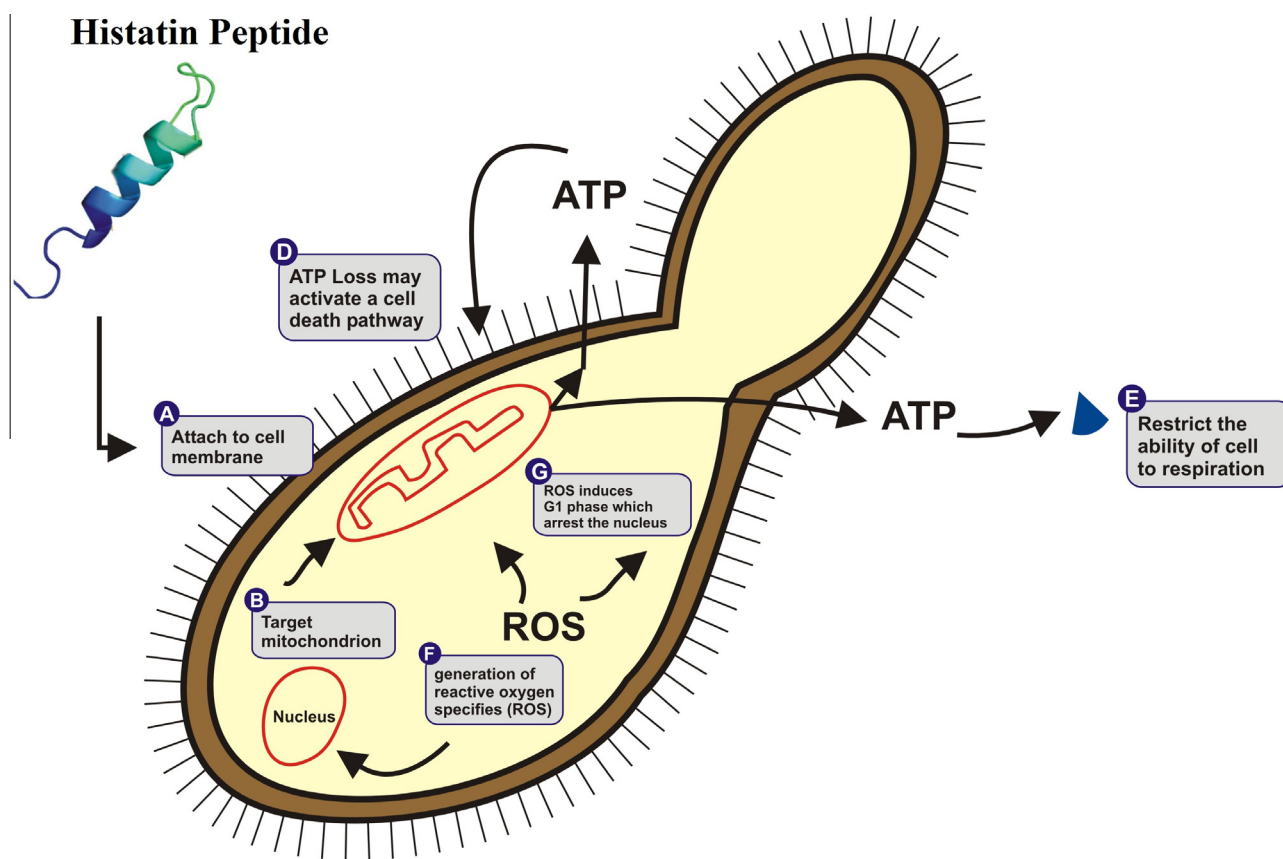
et al., 2008). In comparison, the epithelial growth factor (EGF) and nerve growth factor (NGF) have been reported less stable in terms of wound healing compared to histatins (Oudhoff et al., 2009; Sun et al., 2009).

In recent years, certain valuable researches concerning the effects of histatin on *Candida albicans* have been performed and reported (Tsai et al., 1996; Tsai and Bobek, 1997a; Edgerton et al., 1998). It has been investigated and found that all three major types of histatin peptides (Histatin-1, Histatin-3 and Histatin-5) have the capability to inhibit and kill candidal organisms however, varies in their abilities to kill blastoconidia and germinated cells. In comparison, Histatin-5 has been reported as most active, Histatin-3 being moderately active and His-1 being least active (Xu et al., 1991). Due to this pharmacological nature, histatin is attractive candidate for therapeutic products. The reported mechanism by which histatin acts as fungistatic and fungicidal agent includes disruption of plasma membrane, which leads to the loss of intracellular components (Oppenheim et al., 1988). It was also found that histatins were also effective in killing of yeast by damaging its membrane (due to release of potassium) by binding to the Trk1 potassium transporter and hence loss of intracellular, and azole-resistant fungal species (Fig. 1) (Tsai and Bobek, 1997b; Swidergall and Ernst, 2014). In acquired immuno deficiency syndrome (AIDS), the amount of histatin was found to be decreased and hence it is suggested that they can be used as a biomarker for diagnosis (Khan et al., 2013).

Antimicrobial potential of histatin peptides has been discussed above. This can better be explained by knowing its structure. His-3 and His-5 have capacity to bind to metals, due to the presence of reactive N-terminal (Tay et al., 2009).

Table 3 Histatin Family with their proteolytic fragments (Khurshid et al., 2015).

Human histatins	Sequences
Histatin 1	DSpHEKRHHGYRRKFHEKHHSHREFPFYGDYGSNYLYDN
Histatin 3	DSHAKRHHGYKRRKFHEKHHSHRGYRSNYLYDN
Histatin 5	DSHAKRHHGYKRRKFHEKHHSHRGY
<i>Proteolytic fragments in saliva</i>	
Histatin 2	RKFHEKHHSHREFPFYGDYGSNYLYDN
Histatin 4	KFHEKHHSHRGYRSNYLYDN
Histatin 6	DSHAKRHHGYKRRKFHEKHHSHRGYR
Histatin 7	RKFHEKHHSHRGY
Histatin 8	KFHEKHHSHRGY
Histatin 9	RKFHEKHHSHRGYR
Histatin 10	KFHEKHHSHRGYR
Histatin 11	KRHHGYKR
Histatin 12	KRHHGYK

**Figure 1** Mechanism of action of human histatin peptides against *Candida albicans*.

Herein, this N-terminal has high potential to bind with the metals especially copper and nickel, thus generating reactive oxygen species which damages membranes of cell organelles, also may damage the DNA and hence leads to the fungal and bacterial cell death (Harford and Sarkar, 1997; Grogan et al., 2001). *Bacteriodes gingivalis*, one of the important micro floras of the oral cavity is associated with the destruction of the periodontium as it produces proteases (Gusman et al., 2001). In addition, effects of histatin peptides on secreted proteases and clostripain have been investigated (Imatani et al., 2000).

It was concluded that Histatin-5 inhibited the activity of proteases and clostripain (*clostridiopeptidase B*, *Clostridium histolyticum proteinase B*). It binds to the protease active sites making non-covalent complexes, therefore playing a key role in preventing the periodontitis (Nishikata et al., 1991). The antiviral property of histatins was also investigated; however, little is known about it. There is need of further research to better understand the three dimensional structure of histatin and to rule out which salivary polypeptide is responsible for antiviral activity (Hardestam et al., 2008).

4. Histatin and advances in bio-dental research

The ability of histatin in inhibiting fungal infections can be observed in patients suffering from xerostomia, since they are most susceptible of acquiring infections. Therefore histatins can be used in medicines to help in preventing such infections (Tati et al., 2014). In denture wearers where their immunity is compromised it was observed that they were prone to opportunistic pathogens such as *C. albicans* (Farah et al., 2000; Samaranayake and Samaranayake, 2001). Experimentation was done to see the effect of growth of candidal species on polymethyl methacrylate (PMMA) and it was demonstrated that histatins inhibited the growth of these microorganisms (Vukosavljevic et al., 2012). Thus, this can be utilized as a powerful tool in prevention and as a therapeutic agent in minimizing the possibilities of getting fungal infections (Peters et al., 2010; Iqbal and Zafar, 2016).

There are a number of potential clinical applications for using histatin peptides as a therapeutic agent. As previously mentioned, histatin peptides are playing an effective role in binding to hydroxyapatite crystals. Familiar with this phenomenon, it can be used as a reagent in materials to prevent abrasion and wearing off enamel (Yin et al., 2003; Ullah and Zafar, 2015). Recently, the prime functions of histatins in the field of peptidomics as demonstrated were applied in dental implants for the prevention of implantitis that is considered as one of the important causes of failure of dental implants (Holmberg et al., 2013). The surface of titanium implant was modified by conjoining antimicrobial with titanium-binding peptides, which resulted in decreased formation of biofilm (Yoshinari et al., 2010) and reduced adherence of microorganisms such as *Pseudomonas gingivalis* on the sub gingival and supra gingival surfaces of implants (Yoshinari et al., 2010; Yeo et al., 2012).

5. Conclusion and recommendations

Histatin peptides are the key elements of antimicrobial peptides that have bactericidal and anti-inflammatory activities (Helmerhorst et al., 2001). One of the drawbacks of histatin to be used in drugs as a therapeutic agent was that they were not stable against proteolysis (Sabatini et al., 1989). After studying metallo-peptide nature of histatin and its morphology, we are very well aware of the fact that they have potential to form bond with metal ions especially with copper and nickel at a physiological pH (Cabras et al., 2007). This property can greatly be used in terms of histatin binding to these metals. In addition, histatin becomes more stable and resistant to proteolysis hence, can be used in drugs to combat against oral pathogens (Zawisza et al., 2014).

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