Histatin peptides: Pharmacological functions and their applications in dentistry

Zohaib Khurshid, Shariq Najeeb, Maria Mali, Syed Faraz Moin, Syed Qasim Raza, Sana Zohaib, Farshid Sefat, Muhammad Sohail Zafar

Department of Dental Biomaterials, College of Dentistry, King Faisal University, Al-Ahsa, Saudi Arabia
School of Dentistry, University of Sheffield, Sheffield, UK
Department of Endodontics, Fatima Jinnah Dental College, Karachi, Pakistan
National Centre for Proteomics, Karachi University, Pakistan
Institute of Research and Consulting, King Faisal University, Al-Hofuf, Saudi Arabia
Department of Biomedical Engineering, King Faisal University, Al-Hofuf, Saudi Arabia
Department of Medical Engineering, University of Bradford, Bradford, UK
Department of Restorative Dentistry, College of Dentistry, Taibah University, Al Madinah Al Munawwarah, Saudi Arabia

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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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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; Kotá et al., 2015) or by pathologically by diseased tissues such as ameloblastoma (Jamb 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., 1990; Van et al., 1997), histatin family (Oppenheim et al., 1990; Vitorino et al., 2004; Gorg et al., 2004; Wittmann-Liebold et al., 2006). The common variants of natural histatins found in saliva are Histatin-1 (38 amino acids; Mw ~ 4929 Da), histatin-3 (32 amino acids; Mw ~ 4063 Da) and Histatin-5 (24 amino acids; Mw ~ 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 (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).

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 µg/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 ~ 4929 Da), histatin-3 (32 amino acids; Mw ~ 4063 Da) and Histatin-5 (24 amino acids; Mw ~ 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).

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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...
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 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). Histatin-1 and Histatin-2 are both effective in killing of 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 immune 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).
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). *Bacteroides gingivalis*, one of the important microfloras 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).

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**Figure 1** Mechanism of action of human histatin peptides against *Candida albicans.*
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; Samararayake and Samararayake, 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 dential 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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