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REVIEW

Antibacterial effect of bestatin during periodontitis

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Abstract Periodontitis is a polymicrobial disease inciting inflammatory destruction of the tooth-supporting tissues, i.e., periodontium. The initiation of this infectious disease is ascribed to the formation of subgingival biofilms. These biofilms cause stimulation of myriad of chronic inflammatory reactions by the affected tissue. The Gram-negative anaerobe *Porphyromonas gingivalis* is commonly found as part of the microbiota of subgingival biofilms, and is involved in the occurrence of the disease. *P. gingivalis* possesses numerous virulence factors supporting its survival, regulating its communication with other species in the biofilm, degrading host tissues. *Fusobacterium nucleatum* is pivotal for formation of biofilm and promotes growth and invasion properties of *P. gingivalis*. Bestatin is an aminopeptide inhibitor, produced by actinomycetes. It possesses antibacterial properties against *P. gingivalis* and *F. nucleatum*. The following review focuses on action of bestatin on the mentioned bacteria.

Keywords bestatin, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, periodontitis, biofilm

Introduction

Periodontitis can be elaborated as a chronic inflammation in which the resident cells and preformed mediators lead to induction of leukocyte infiltration and progressive destruction of the tissues anchoring tooth. This results as an interaction between host cells, bacterial products and the mediators in disease-susceptible individuals (Araújo et al., 2015). If the disease is not treated, it results in degradation of ligamentous supporting structure along with resorption of alveolar bone thus leading to tooth mobility and eventually tooth loss. Chronic periodontitis is regarded as major etiology of tooth loss (Sippert et al., 2015). It is caused by the bacteria in the biofilm on the tooth surface. There are a number of molecules released from the biofilm which activate the inflammatory response. The process activated by these molecules includes migration of neutrophil, monocytes and lymphocytes and activation of bone resorbing osteoclasts (Boström et al., 2015).

Periodontitis is known to humans since middle age. It was prevalent after the domestication of plants and animals during the Neolithic society. This period was a landmark for change in oral microbiota, there was increase in frequency of periodontopathogens, which were less in hunter-gatherer societies. Though the periodontopathogen are the causative agents, the progression of the disease is determined by the host immune response, including a myriad of cells namely neutrophilic polymorphonuclear leukocyte, macrophages, lymphocytes and fibroblasts (Gonzales, 2015).

Biofilms are attached to tooth surface and approximately 500 species of microorganisms can be present in the biofilm. The species most prominently related with periodontitis are *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*. Among these three species which form the red complex, *P. gingivalis* plays a pivotal role harmonizing the virulence of the biofilm thus causing inflammation of the periodontium (Bao et al., 2014). *P. gingivalis* has been designated as a keystone pathogen, similar to the keystone supporting stone at the apex of an arch (Hajishengallis, 2014). *P. gingivalis* is a gram-negative, non-motile, short rod or coccobacillus. It thrives in areas comprising complex nutritional arrangement and reduced oxygen tension like subgingival region (Lamont and Jenkinson, 2000). *P. gingivalis* were first isolated by Burdon in the year 1928 and since then they have been isolated with periodontal inflammation. It is observed in adolescents and adults and its level has been related to increasing age (Dahlén, 1993).

The pathogenesis of *P. gingivalis* is due to a number of virulence factors enlisting fimbriae, lipopolysaccharide, outer

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membrane vesicles, cysteine proteases, capsules, hemagglutinin, adhesion and colonization of the bacteria, production of proteolytic enzymes and outstripping the immune system (Jauregui et al., 2013; Bao et al., 2014). Cysteine protease (gingipains) includes arginine-specific proteinases RgpA and RgpB and the lysine specific proteinases Kgp (Bao et al., 2014).

Under strict anaerobic condition, *P. gingivalis* is primarily dependent on gingipains for utilizing free amino acids as a source of carbon and nitrogen. They are pivotal in evading host defenses as they degrade antibacterial peptides like neutrophil-derived α-defensins, complement factor, including C3 and C4, T cell receptor like CD4 and CD8 (Bao et al., 2014). Gingipains along with an outer membrane protein HA-Ag2 causes nutrient acquisition, inactivation of cytokines and components of the complement system, cleavage of host cell surface receptor and signaling via protease-activated receptors. Kgp cleaves a number of connective tissue and plasma constituents including immunoglobulins, fibronectin, plasma kallikrien, fibrinogen, iron, heme, hemoglobin-transporting protein and peptidase inhibitor. This facilitates bleeding and vascular permeability along with heme and iron uptake by the bacterium (Cutler et al., 1995; de Diego et al., 2014).

Bestatin

Bestatin is an aminopeptide inhibitor. It is produced by actinomycetes. The structure of bestatin is [(2S,3R)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine. The molecular formula is $C_{16}H_{24}N_2O_4$ and is obtained as colorless fine needles. It is amphoteric in nature and has single free amino, hydroxyl and carboxyl group (Suda et al., 1976). Bestatin inhibits exopeptidase and endopeptidase inhibitors including trypsin, plasmin, papain, chymotrypsin, elastase, acid protease and thermolysin (Umezawa et al., 1976). Bestatin is an inhibitor of aminopeptidase B and leucine aminopeptidase, first reported in 1970s (Nakamura et al., 1976). Aminopeptidase B is an exopeptidase obtained from rat liver. It causes hydrolysis of N-terminal bond containing N-terminal arginine or lysine (Umezawa et al., 1976). Leucine aminopeptidase and aminopeptidase B catalyze cleavage of N terminus of proteins and peptides (Labbé et al., 2001).

Bestatin was discovered in the culture supernatant of Streptomyces olivoreticuli. It is a slow binding inhibitor of aminopeptidase and inhibits its activity both in the fluid and on the cell surface. It also inhibits the proliferation of leukemia-lymphoma cell lines in vitro and it improves the survival rate of patients suffering from acute non-lymphocyte leukemia. In a study it was observed that bestatin treatment inhibited interleukin (IL)-6, CXCL8/IL-8, CCL3/MIP-1α in patients with sarcoidosis. The study concluded that it inhibits production of pro-inflammatory cytokines and stimulates production of anti-inflammatory cytokines (Lkhagvaa et al., 2008). It also augments delayed type hypersensitivity to sheep red blood cells. It enhances immune resistance to cancer and suppresses growth of tumor which is a result of second inoculation of Ehrlich carcinoma cells. It is also found to enhance the effects of bleomycin during the treatment of Ehrlich carcinoma (Umezawa et al., 1976). Bestatin exhibits mitogenic effects on lymphocytes. It stimulates proliferation of T cell through the activation of macrophages. It was observed that the high concentration of bestatin exhibited high mitogenic response on B cell and partly T cell (Ishizuka et al., 1980).

Aminopeptide N or CD13 is observed to be a cardinal regulator of angiogenesis. In a study by Mishima et al. the effect of a number of CD13 antagonists on vascular endothelial capillary-like tube formation was tested. Among all the antagonists tested only bestatin significantly inhibited activity of CD13. It interfered in the capillary tube formation of primary endothelial cells. It was concluded that bestatin downregulates reactivity of endothelial cells to stimuli of angiogenesis and modulates a number of angiogenesis-related genes including vascular endothelial growth factor (Mishima et al., 2007). In another study Hossain et al. evaluated role of bestatin in the retina of streptozotocin-induced diabetic mice. CD13 was observed to be expressed in neovascularization induced by hypoxia in non-diabetic rats. It was observed that bestatin treatment inhibited the symptoms including retinal vascular permeability and leukostasis. The treatment was even effective in inhibiting expression of CD13 in retina and protease degrading extracellular matrix (Hossain et al., 2016).

A study by Wang et al. (2016) observed that bestatin caused autophagic cell death in both metastatic and non-metastatic prostate cancer cells as well as inhibited prostate cancer cells proliferation and inhibited the cell migration and invasion in prostate cancer cells by decreasing aminopeptidase N expression. The same results have been observed for renal cell carcinoma. Liu et al. concluded that bestatin inhibits proliferation, migration and invasion of renal carcinoma cells (Liu et al., 2015).

Effect of bestatin on periodontopathogen

Bestatin selectively inhibits growth of *P. gingivalis* and is indifferent to other oral microorganisms. This effect on periodontopathogen is of interest as subgingival indigenous microflora, which is important for providing protection against opportunistic pathogens. Bestatin affects *P. gingivalis* by inhibiting uptake of peptides and amino acid which are the nutrient sources for the survival and multiplication of the bacteria. At the concentration of 35 μg/mL, bestatin weakly inhibited epithelial cell proliferation thus prevented excessive growth of periodontal pocket epithelium. It is not cytotoxic, thus may be a potential therapeutic molecule for treating periodontitis locally (Labbé et al., 2001). Inhibitory effects of bestatin on *P. gingivalis* were first reported by Grenier in the year 1992. It was observed that bestatin had strong inhibitory effect on the growth of *P. gingivalis* but it did not exhibit any

effect on the cell-associated proteolytic activity (Grenier, 1992).

Grenier and Michaud in 1994 evaluated role of bestatin on inhibition of *P. gingivalis*. It was observed that there was complete inhibition of 11 strains of *P. gingivalis* by bestatin at the concentration of 2.5 μg/mL and half inhibition at the concentration of 0.5 μg/mL, thus stating that the effect was dose concentration related. It was also observed that bestatin had bacteriostatic but not bactericidal effect. It was noted that bestatin can confer protection against candida albicans and hysteria monocytogenes infections and the effect was observed to be due to its stimulatory effect on neutrophil (Grenier and Michaud, 1994).

A study claimed that bestatin along with other proteinase inhibitor attenuated visible leaky site formation and inhibited gingipain RgpA induced responses in the hamster cheek pouch (Rubinstein et al., 2001). Kitano et al. tested bestatin activity on *P. gingivalis* and confirmed its activity by stating the bestatin strongly exhibits antibacterial activity. It was also noted that its effect was superior to other inhibitor that were tested (Kitano et al., 2001). In an experiment Yoshika et al. evaluated protein degradation by *P. gingivalis* using Flurophore-labeled substrates, fluorescent bovine serum albumin. Bestatin was observed to reduce degradation of bovine serum albumin (Yoshioka et al., 2003).

Fusobacterium nucleatum is one of the most studied bacteria implicated in periodontal inflammation. It is a gram negative anaerobic bacteria belonging to bacteriodaceae family (Signat et al., 2011). Rogers et al. tested efficacy of bestatin on *F. nucleatum* and stated that Bestatin inhibited growth of *F. nucleatum* in a medium in which peptidase activity was a nutritional necessity (Rogers et al., 1998). *P. gingivalis*, *F. nucleatum* along with *Streptococcus gordinii* are the three preeminent bacteria for the formation and augmentation of the virulent biofilm (Wang et al., 2015). *F. nucleatum*, *P. gingivalis* and *Aggregatibacter actinomycetemcomitans* (cardinal periodontopathogen) interact with each other and enhance the invasion and adherence into the human gingival epithelial cells (Li et al., 2015). In both the situations mentioned above, *F. nucleatum* augments invasion, adherence and proliferation of *P. gingivalis* and *A. actinomycetemcomitans* (Li et al., 2015; Wang et al., 2015).

Conclusion

From the above data it can be concluded that bestatin can be an effective treatment modality against periodontitis as both *P. gingivalis* and *F. nucleatum* are highly virulent. The prodigious fact regarding bestatin is that it is bacteria specific in its action. This property will be beneficial to save commensal bacteria and act specifically on virulent ones. I was adept in finding literature linking the topic but would like to terminate on the point that there is scarcity of literature and further clinical trials and studies are warranted to corroborate on the topic. I am sure the drudgery put in will not go in vain.

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Compliance with ethics guidelines

This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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