The plasminogen activation system in periodontal tissue (Review)

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Abstract. The plasminogen activation system (PAS) plays an essential role in tissue proteolysis in physiological and pathological processes. Periodontitis is a chronic infection associated with increased proteolysis driven by plasminogen activation. In this comprehensive review, we summarise the effects of PAS in wound healing, tissue remodelling, inflammation, bacterial infection, and in the initiation and progression of periodontal disease. Specifically, we discuss the role of plasminogen activators (PAs), including urokinase PA (uPA), tissue-type PA (tPA), PA inhibitor type 1 (PAI-1) and 2 (PAI-2) and activated plasminogen in periodontal tissue, where their concentrations can reach much higher values than those found in other parts of the body. We also discuss whether PA deficiencies can have effects on periodontal tissue. We conclude that in periodontal disease, PAS is unbalanced and equalizing its function can improve the clinical periodontal tissue condition.

Contents

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1. Introduction

The plasminogen activation system (PAS) is a complex enzymatic cascade, where individual components activate or deactivate the final product of that cascade, plasmin. The process of plasminogen transformation into plasmin is initiated by two activators, the urokinase plasminogen activator (uPA) and the tissue-type PA (tPA), which are controlled by PA inhibitor type 1 (PAI-1) and type 2 (PAI-2) and the protein, nexin (1,2). Moreover, urokinase can be present as a free enzyme or can bind to the cell surface by the uPA receptor (uPAR) (3,4).

The physiological significance of the urokinase plasminogen system results from the widespread role of plasmin in physiology and pathology. Plasmin degrades fibrin into soluble peptides and is responsible for the elimination of its excess from the circulatory system and tissues. In addition, it actively hydrolyses numerous proteins, including, laminin and type IV collagen, which are the basic structural elements of the extracellular matrix and vascular basement membrane. Their decomposition takes place both in consequence of the direct action of plasmin and indirectly, due to its activating the cascade of matrix metalloproteinase (MMP) precursors, such as MMP-1 (collagenase precursor), MMP-3 (stromelysin precursor) and MMP-9 (gelatinase B precursor) (5). Under *in vivo* conditions, plasminogen and plasmin are present in the blood circulation and in the matrix on the cellular surface (6). The activation of plasminogen by tPA is mainly related to processes of the dissolving of fibrin in the vascular injury region. On the other hand, uPA acts in the extracellular space, activating plasminogen, initiating a proteolytic cascade reaction there. The intensive spot concentration of urokinase on the cell surface leads to the increased formation of plasmin and focused proteolytic activity, enabling cell migration (6-9). Moreover, uPA/uPAR and extracellular matrix vitronectin or integrins can adhere cells to the extracellular matrix. Since the uPA/uPAR complex can be internalized, degraded by

endocytosis in cooperation with the transmembrane low-density lipoprotein-related-receptor (LPR) under certain conditions, it creates some areas free of these complexes. Thus, the newly synthetized uPA/uPAR complex will 'seek' vitronectin and integrins intensifying cell motility. These pro-adhesive pro-motility urokinase properties are not related to its proteolytic activity (6,10). By interaction with the specific receptor located on the cellular surface, urokinase acquires unique chemokine-like characteristics and regulates migration and the related processes of changes in cell shape, adhesion, chemotaxis and invasion of the extracellular matrix (8).

The overexpression of PAs (tPA and uPA) has been found in numerous human diseases (1). PAI-1 is the main physiological inhibitor of uPA and tPA, belonging to the serpin superfamily (serine proteinase inhibitors), and it is produced as a single chain glycoprotein, composed of 402 amino acids with a molecular weight of 45 kDa (11). Its essential function in the organism is to regulate PA activity and, consequently, the formation of plasmin (Fig. 1). Thus, PAI-1 is an important element of systemic homeostasis, which influences the physiological equilibrium of coagulation and fibrinolytic systems under *in vivo* conditions. Its abnormal elevated expression is observed in a number of pathogenic processes, including atherosclerosis, coronary artery disease, sepsis, multiple sclerosis, pulmonary and renal fibrosis, obesity and insulin resistance, as well as neoplastic disease (2,12-14).

PAI-1 is a highly specific inhibitor of tPA and uPA; however, it is not stable and converts itself into the latent form, with a half life ranging between $t_{1/2}$=1-2 h, which is unable to interact with target molecules. This presents an obstacle in using wild-type PAI-1 as a hemostatic drug (20,21). Therefore, PAI-1 mutants have been produced in the past which delay or prevent its conversion into the latent form (Fig. 2), extending its half-life to approximately $t_{1/2}$=6-170 h (16-18). The most stable is the VLHL PAI-1 mutant with an engineered disulphide bridge (Cys197-Cys355); without disrupting the molecular structure it prolongs its half-life to approximately $t_{1/2}$=700, enabling plans for its possible therapeutic utilization (15). Indeed, VLHL PAI-1 has successfully been used to shorten the total time of bleeding and total blood loss in mice (22,23).

On a cellular level PAI-1 inhibits the consecutive stages of cell migration both by direct blockage of the proteolytic activity of urokinase and by induction of the internalization of the uPA-uPAR complex and suppression of its chemokine-like and adhesion-like effect (19).

In molecular interactions PAI-1 inserts arginine 369 into the specificity pocket of uPA or tPA, preventing the reaction of activators with plasminogen. Similar blocking can be accomplished by small molecules or polypeptides. Thus, there is a search for such small molecule inhibitors, which can block PA activity as the natural inhibitor, PAI-1 does (24-26). It seems feasible to seek these molecules among dietary components that pass through the oral cavity.

2. Plasminogen activation system in periodontal disease

PAs have been found in the oral mucosa (27), saliva (28) and gingival crevicular fluid (GCF) (29) in 100-fold greater concentration than in plasma. The activity of PAs was observed at the site of desquamation of the junctional epithelium in healthy tissue and post-treatment, in which there were residual inflammatory cells. This site-specific pattern is greatly altered during periodontal disease (29), as described below.

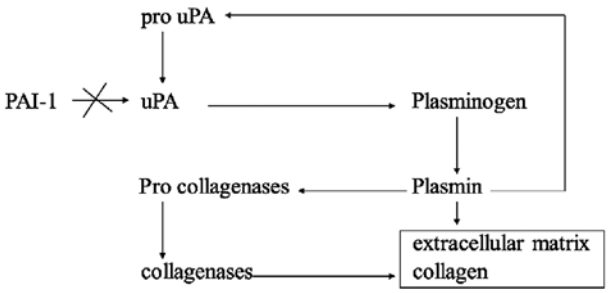

Figure 1. Pro-urokinase plasminogen activator (uPA) and tissue-type plasminogen activator (tPA) are synthesized and activated by catalytic amounts of plasmin. Both activators cleave plasminogen to active plasmin, that degrades proteins of the extracellular matrix or activates other proteases.

3. Wound healing

Wound healing in periodontal tissue is a complex interrelationship between epithelial and connective tissue, affected also by contact with saliva and crevicular fluid. Wound healing in the periodontal connective tissue as a new attachment or re-attachment requires different types of connective tissue matrices, but initially it is similar to the other types of connective tissue and proceeds with provisional matrix formation (30), of which fibrin is a major constituent (31).

It has been well documented that during the early phase of wound healing (1-3 days), PAI-1 and PAI-2 are strongly associated with infiltrating inflammatory cells around the fibrin clot in the gingival sulcus, accompanied by the presence of uPA and the absence of tPA in healing wounds (32,33). During the next phase (granulation tissue formation) of wound healing, uPA, PAI-1 and PAI-2 are observed in monocytes, macrophages and fibroblasts (32,33). PAI-1 has been shown to be an important factor in inflammation-induced macrophage migration, which is essential for defense functions (34). uPA additionally has been observed in endothelial cells of newly formed blood vessels (32). It has been suggested that plasminogen activation plays a role in the migration of keratinocytes (35) and fibroblasts (33,36), as well as tissue remodeling in the wound healing (32) of periodontal tissue. The role of PAS in the regulation of fibronectin proteolysis and fibroblast apoptosis and a potential role of transforming growth factor-β1 (TGF-β1)/PAI-1 in promoting (myo)fibroblast survival in chronic fibrotic disorders has also been pointed out (37). Fibronectin, one of the extracellular matrix components, is a substrate for PA (38). Additionally, it can be the substrate for multiple host and bacterial proteinases found in inflamed periodontal sites. The smaller fragments of fibronectin (30 and 45 kDa) are the most potent inflammatory inducers, as they have been shown to dose-dependently increase the secretion of tumor necrosis factor-α (TNF-α), and interleukin (IL)-1β and IL-8 by human macrophages (39).

Plasmin also plays a role in bone formation through the activation of TGF-β and the release of insulin-like growth factor from the bone matrix, which stimulate osteoblast proliferation and activity (40). Osteoblasts express both PAs, PAI-1 and a cellular receptor for uPA (41). Due to the influence on

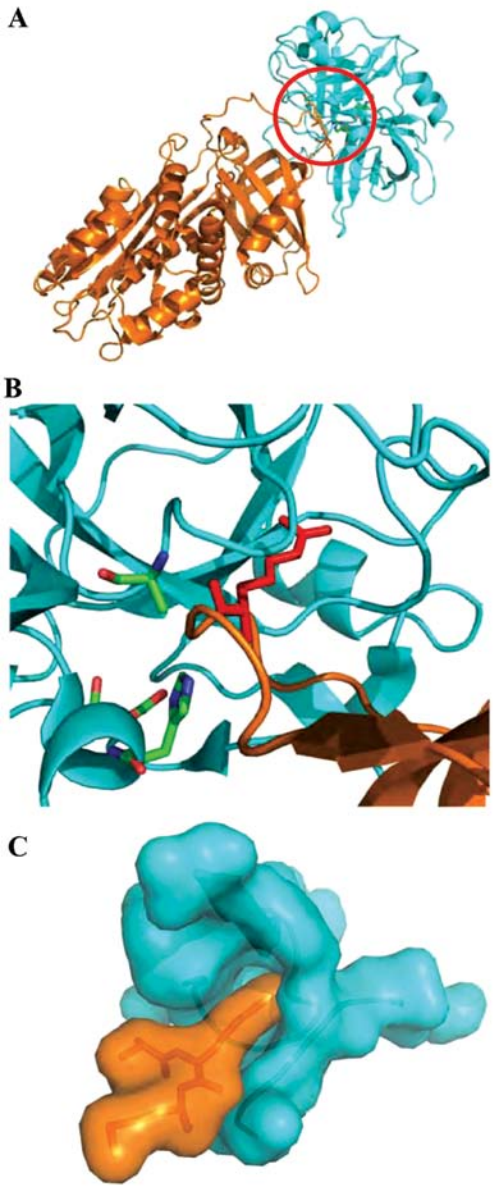

Figure 2. Ribbon model of: (A) active form of plasminogen activator inhibitor-1 (PAI-1) in orange in complex with urokinase plasminogen activator (uPA) in light blue; red circle shows reactive center loop of PAI-1 complexed with uPA; (B) enlarged active site of uPA in complex with PAI-1, active site amino acids (His57, Asp102 and Ser195) shown as stick model colored as follows: green-carbon, blue-nitrogen, red-oxygen; arginine (Arg269) inserted into specificity pocket of uPA is shown as a stick model in red (for clarity hydrogens are omitted). (C) Specificity pocket of uPA and P2, P1, P1' amino acids (red sticks) from the reactive center loop of PAI-1 are shown as surface models (light blue and orange, respectively). The specificity pocket is where the vast majority of uPA inhibitors are bound (16,75-78).

the degradation of non-collagenous components of the bone matrix, plasmin may be important in the resorption phase of bone regeneration (42).

4. Inflammation

From the molecular and cell biology point of view, the periodontium can be considered an organ system composed of two hard tissue (bone and cementum) and two soft tissue (periodontal ligaments and gingiva) components, of which each has a unique tissue architecture and biochemical composition (43,44). The degradation of the extracellular matrix of these components can occur through a number of different pathways, such as MMP activation, reactive oxygen species, cytokine release, prostaglandin synthesis and cell activation, and can modify host responses to inflammatory stimuli, contributing to the imbalance between biofilm and host immune response.

GCF contains both types of PAs, as well as their inhibitors (45); tPA and PAI-2 have been found in GCF in very high concentrations and seem to be predominant components in the gingival sulcus environment (46). Gingivitis (47) and periodontitis (48) have always associated with high levels of those enzymes. The treatment of periodontitis leads to a significant decrease in PAI-2, but not tPA levels (45). In a previous study, differences were observed in tPA/PAI-2 ratios between deteriorating and stable sites in patients treated for periodontitis (49).

Biopsies from gingival tissues of different conditions prepared for *in situ* hybridization and immunochemistry have revealed that in clinically healthy gingiva, both tPA mRNA and antigen were expressed in a thin outer layer of sulcular and junctional epithelium, and similarly but more extensively in chronic gingivitis or early periodontitis. As previously demonstrated, there were no signals or staining for tPA in connective tissue, occasionally in connection with vessels. PAI-2 mRNA was detected in the same sites and intensity as tPA, but also extended toward the connective tissue (50,51).

A previous study demonstrated that there was a significant increase in PA activity and tPA antigen in samples from inflamed gingiva compared to healthy gingiva and with decreasing severity of periodontitis in canines. PAI-1 antigen measured by ELISA was not detected in the collected samples. The authors concluded that PA activity and tPA antigen levels may be used to evaluate the progression of periodontal disease (52).

On the other hand, the progression of periodontitis has been associated with the increase in PAI-1 levels in plasma (53). As PAI-1 is a protein involved in acute-phase response (54) of which periodontitis is one of the components, and hemostatic factor associated with cardiovascular disease development, it has been suggested that periodontitis as a chronic infection may lead to the formation of atherosclerotic lesions (55) and an increased risk of cardiovascular disease (53).

Furthermore, the incubation of periodontal ligament fibroblasts and gingival fibroblasts with platelet-derived growth factor-BB isoform has been shown to increase uPA and PAI-1, but not tPA and PAI-2 levels (56).

5. Microbial infection

Proteases released by periopathogens are responsible for collagen, fibronectin and laminin degradation in periodontal tissues (57). Gingival fibroblasts can be stimulated by a protease of *Bacteroides (porphyromonas) gingivalis* (58), *Porphyromonas endodontalis* (59), lipopolysaccharide (60) and IL-1 (61), to secrete collagenase and PA enzymes (59), but not directly by chemokines (62). In addition, in association with host MMPs, they play an important role in the progression of periodontal disease (63). Bacterial products, such a streptokinase and staphylokinase can activate plasminogen into plasmin by a non-proteolytic mechanism (64). Some bacterial pathogens can induce localised proteolysis, important in tissue

damage and penetration through natural host barriers. During PA production, it is possible to capture the active plasmin on the cell surface and bind human plasminogen, which may be activated into plasmin by host PAs (65,66). Plasminogen bound to bacteria may promote host tissue destruction and invasion even by non-proteolytic bacteria (67). In addition, this mechanism allows bacterial pathogens to migrate from periodontal tissue, through the bloodstream to the various host organs (68).

6. Plasminogen activation system deficiencies

Congenital PAI-1 deficiency is a rare autosomal recessive disorder, characterized clinically by excessive bleeding even with minor cuts or trauma (69). Individuals with PAI-1 deficiency bleed as a result of a hyperfibrinolytic hemorrhage. The primary hemostasis is normal (e.g., a normal thrombus is formed), but it is quickly lysed as there is no inhibitor to moderate tPA plasmin activation (70). Spontaneous bleeding is rarely observed, but moderate hemorrhaging of the gums, knees, elbows and nose can be triggered by very little trauma. Moderate PAI-1 deficiency is manifested with a lifelong bleeding tendency, but severe deficiency can be life-threatening (71). Only two types of mutations have been reported to be associated with PAI-1 deficiency. One is the frame-shift mutation in exon 4 of the PAI-1 gene, resulting in a truncated non-functional protein. The other concerns single nucleotide polymorphisms (SNPs) of Ala15Thr and Val17Ile mutations in the signal peptide. These cause reduced PAI-1 secretion, leading to the deficiency at a time and place where it is needed (72). PAI-1 deficiency can be treated with systemic or topical applications of PAI-1 with an extended half-life (73) or with a 5- to 7-day course of oral tranexamic acid or ε-aminocaproic acid (69).

In their study, El Darouti *et al* (74) reported a case of ligneous conjunctivitis and ligneous periodontitis in association with plasminogen deficiency, based on the decreased serum activity of this enzyme. The predominant plasminogen gene mutation in patients suffering from the disease is the point mutation, Lys19Glu. Topical plasminogen from fresh or frozen plasma in the form of ointments holds some promise as a treatment for the disease. However, impaired extracellular fibrinolysis and chronic mucosal pseudomembranous lesions due to subepithelial fibrin deposition and inflammation most likely will be treated with gene therapy. To our knowledge, there are no studies available on uPA and tPA deficiencies in the pathology of the oral cavity.

7. Inhibitors

The associations between diet and periodontal disease seem to be very interesting in terms of the utilization of nutrients which are natural inhibitors of PAS. PAI-1 is produced by adipocytes and stromal cells surrounding the adipocytes and its level increases, particularly in the abdominal area adipose tissue (79). There are some studies suggesting an association between periodontal disease and obesity (80), particularly in the case of visceral fat, which leads to hepatic steatosis (81).

Recent studies have revealed better periodontal conditions in vegetarians (82), who consume a large amount of soy food. They manifest in the improvement of such clinical parameters, as lower probing pocket depth (PPD), bleeding on probing (BOP) and periodontal screening index (PSI) The highest two quintiles of total soya intake have been associated with a 32-35% reduction in the odds of developing periodontal disease (83). Soybeans are a unique source of isoflavones (genistein, daidzein) which can inhibit epidermal growth factor (EGF)-stimulated urokinase production (84). Similar daily consumption of other polyphenols, such as catechins in green tea has shown to have a positive impact on periodontal clinical parameters (85). One of the suggested mechanisms is the activation of kinase-signaling cascades that suppress PAI-1 expression (86). Epigallocatechin-3-gallate (EGCG), a major green tea catechin, has been shown to downregulate TNF and IL-6 expression, leading to a decrease in osteoclast number and activity in rats (87). EGCG also inhibits CC chemokine ligand 11 (CCL11) production in human gingival fibroblasts, related to Th2 cell migration (88).

The other well documented component of food with beneficial properties is a popular spice, curcumin. It may have therapeutic potential in periodontal disease (89), possibly through the upregulation of uPA, which is instrumental in promoting fibrynolysis and cell migration during wound healing (90).

8. Conclusion

Periodontal disease is a chronic, opportunistic infection, caused by an imbalance between biofilm-forming bacteria and the host immune response. Even in clinically healthy gingiva nutrophiles and lymphocytes are still present and endothelial cells reactions are stimulated by adhesins. Acute inflammation is always associated with bleeding, redness and swelling of the gingival tissue and with an increased leakage of crevicular fluid. Endogenous mediators of inflammation, such as PAS, are crucial for this phase of inflammation. However, the components of PAS are also detected in all periodontal cells in the chronic and healing phase of inflammation, which may indicate their complex influence on the periodontium. The deficiency of plasmin or PAI-1 causes spontaneous bleeding and the destruction of periodontal supporting tissue, and can be treated with topic or systemic supplementation. On the other hand, natural inhibitors of PAI-1 can improve the clinical periodontal tissue condition. Knowledge of the complex interactions of proteins in disease is derived mostly from animal models. Proteins of different organisms frequently differ from each other in a surprising manner. For example, mouse uPA and human uPA are very similar to each other, while rat uPA differs significantly from that of mice and humans. Thus, it is important to remember that conclusions derived from animal studies must take into consideration the limitations of the selected animal model and its applicability to humans.

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