

Roles of secretory leukocyte protease inhibitor amniotic membrane in oral wound healing

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ABSTRACT

Secretory Leukocyte Protease Inhibitor (SLPI) is serine protease inhibitor. Secretory Leukocyte Protease Inhibitor is a protein found in secretions such as whole saliva, seminal fluid, cervical mucus, synovial fluid, breast milk, tears, and cerebral spinal fluid, as in secretions from the nose and bronchi, amniotic fluid and amniotic membrane etc. These findings demonstrate that SLPI function as a potent anti protease, anti inflammatory, bactericidal, antifungal, tissue repair, extra cellular synthesis. Impaired healing states are characterized by excessive proteolysis and often bacterial infection, leading to the hypothesis that SLPI may have a role in the process. The objectives of this article are to investigate the role of SLPI in oral inflammation and how it contributes to tissue repair in oral mucosa. The oral wound healing responses are impaired in the SLPI sufficient mice and matrix synthesis and collagen deposition are delayed. This study indicated that SLPI is a povital factor necessary for optimal wound healing.

Key words: *Secretory leukocyte protease inhibitor, amniotic membrane, oral wound healing*

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INTRODUCTION

Secretory leukocyte protease inhibitor (SLPI) is a serine protease inhibitor has recently been shown to be capable of a range of immunological functions, including anti-inflammatory and anti-microbial activity. At tissue level, the ability of this ptoein to counteract the excessive degradation of functional and structural proteins such as collagen and fibronectin, as seen in impaired cutaneous wound has led to further inquiry into its degree of involvement within the wound healing process. Moreover, since wound healing in the oral cavity occurs more rapidly and with minimal scarring compared to the skin, molecules in the oral cavity, such as SLPI, may contribute to this differential healing response.^{1,2,3}

The inflammatory process in wound healing includes the release of several mediators such as chemoattractants, cytokines and proteinases that regulate the adhesion of molecules, and the processes of cell migration, activation and degranulation. The characteristic destruction of tissue in inflammatory diseases is to a large extent mediated by an excess of neutral serine proteinases. States of impaired healing are characterized by excessive proteolysis and often bacterial infection, leading to the hypothesis that SLPI may also have a role in this process.⁴

Human SLPI is an 11.7–12 kDa cationic protein and a member of the innate immunity-associated proteins.^{1,2,3} It is a nonglycosylated, highly basic, acid-stable, cysteine-

rich, 107-amino acid, single-chain polypeptide.⁵ The SLPI gene, along with the elafin gene, is a member of the trappin gene family. The products of this family are characterized by an N-terminal transglutaminase domain substrate and a C-terminal four-disulfide core.⁶

SLPI is produced by neutrophils, macrophages, beta-cells of pancreatic islets, epithelial cells investing the renal tubules, acinar cells of parotid and submandibular glands, acinar cells of submucosal glands, and epithelial cells lining mucous membranes of respiratory and alimentary tracts.^{7,8} SLPI was originally isolated from parotid saliva and has been detected in a variety secretions such as whole saliva, seminal fluid, cervical mucus, synovial fluid, breast milk, tears, and cerebral spinal fluid, as in secretions from the nose and bronchi, etc.⁵ The SLPI gene was found to be expressed in lung, breast, oropharyngeal, bladder, endometrial, ovarian, and colorectal carcinomas, and SLPI detection is correlated with poor prognosis. SLPI is also found in neurons and astrocytes in the ischemic brain tissue.¹ Finally, SLPI was found to play a pivotal role in apoptosis and wound healing.^{2,9} Given that SLPI is a ubiquitous protein, it has received many alternative names, including mucus protease inhibitor, antileukoprotease, bronchial secretory inhibitor, human seminal inhibitor I, cervix uteri secretion inhibitor, and secretory leukoprotease inhibitor.¹ The objectives of this article are to investigate the role of SLPI in oral inflammation and how it contributes to tissue repair in oral mucosa.

Amniotic membrane

Amniotic membrane is the thin membrane that covers the placenta and baby before it is born. It has many properties that make it ideal for use as a transplant material. It was first used as a surgical dressing for skin burn. It is now utilized as a biological dressing for burned skin, skin wound and chronic ulcers of the leg as an adjunctive tissue in surgical reconstruction of artificial vagina and for repairing omphalocheles. It has also been used to prevent tissue addition in surgical procedures of the abdomen and pelvis.¹⁰

Amniotic membrane has been found to facilitate epithelialization, maintain a normal epithelial phenotype, reduce inflammation, reduce scarring, reduce the addition of tissue, reduce vascularisation, a number of cytokines, growth factors such as IL-4, IL-6, IL-10, EGF, FGF, TGF, HGF and 2-macrobilin. Biological active protease inhibitors include SLPI.¹¹

Secretory leukocyte protease inhibitor (SLPI)

The main function of SLPI is to protect local tissue against the detrimental consequences of inflammation. Indeed, a plethora of toxic (inflammatory) products, i.e., serine proteinases, is released from stimulated leukocytes during inflammation, and subsequent degradation of the tissues ensues. SLPI protects the tissues by inhibiting the proteases, such as cathepsin G, elastase, and trypsin from neutrophils; chymotrypsin and trypsin from pancreatic acinar cells; and chymase and tryptase from mast cells.¹² Based on enzyme kinetic studies, its major physiologic function is probably the inhibition of neutrophil elastase.¹³ Neutrophil elastase as well as mast cell proteolytic enzymes can cause extensive tissue degradation and has been shown to be involved in several diseases, such as cystic fibrosis, non-cystic fibrosis bronchiectasis, emphysema, acute respiratory distress syndrome, chronic bronchitis, and bacterial pneumonia.¹²

It is generally postulated that the balance between proteinases and antiproteinases is a prerequisite for the maintenance of tissue integrity. Indeed, it is shown that cleavage of SLPI results in increased tissue damage.¹⁴

SLPI also shields the tissues against inflammatory products by down-regulating the macrophage responses against bacterial lipopolysaccharides (LPS). LPS seem to induce SLPI production by macrophages directly or by way of interleukin-1 β (IL-1 β), tumor necrosis factor alpha, IL-6, and IL-10.¹⁵ Secretory leukocyte protease inhibitor in turn inhibits the downstream portion of the nuclear factor κ B (NF- κ B) pathway by protecting I- κ B (inhibiting factor of NF- κ B) from degradation by the ubiquitin-proteasome pathway. Thus, SLPI renders macrophages unable to release proinflammatory cytokines and nitric oxide.¹² Ding *et al.*¹⁶ point out that the inhibitory effect of SLPI on macrophage responses may be due to its blockade of LPS transfer to soluble CD14 (receptor of macrophages) and its interference with the uptake of LPS from LPS-soluble CD14 complexes by macrophages. Taggart *et al.*¹⁷ suggest that SLPI attenuates macrophages' responsiveness by inhibiting the LPS pathway through suppression of NF- κ B

and activation of CCAAT β enhancer-binding protein-transcription. Thus, the accumulation of SLPI in the local tissue environment may represent an intrinsic feedback inhibition mechanism.

Although there are only a few published studies pertinent to this field, recent scientific evidence suggests that SLPI has broad-spectrum antibiotic activity that includes bactericidal and antifungal properties. In a recent study, Fahey and Wira¹⁸ examined the production of antibacterial factors by uterine epithelial cells from pre- and postmenopausal women. Apical rinse fluids from polarized epithelial cells recovered from women at the proliferative and secretory stages of the menstrual cycle were equally effective in killing *Staphylococcus aureus* and *Escherichia coli*, but those from postmenopausal women were not. SLPI concentrations in apical wash fluids from premenopausal women were significantly higher than those in wash fluids obtained from postmenopausal women. SLPI production correlated with bactericidal activity with respect to menstrual status and culture time. Anti-SLPI significantly decreased bactericidal activity of premenopausal epithelial cell rinse fluids. The endometrial epithelial cell line HEC-1A did not have a bactericidal effect, nor did it produce SLPI. In contrast, HEC-1B cells produced SLPI and a factor that inhibited bacterial growth. It seems that the N-terminal domain is responsible for the dose-dependent bactericidal properties of SLPI against both gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria. Hiemstra¹² showed that the activity of this domain is not as efficient as the one of the intact molecule. Hence, they speculated that a conformational change in the N-terminal domain is induced by the cleavage procedure of the native protein.¹² In addition, suggested that the mechanism of the SLPI-mediated bactericidal activity may include binding of the protease inhibitor to the bacterial mRNA and DNA, but Hiemstra¹² findings proved that this binding is not enough to explain the antibacterial activity of SLPI. The antiprotease domain of SLPI seems to play a crucial role in regulating host defense against infections by inhibiting the elastase-mediated degradation of opsonins and receptors involved in phagocytosis and controlling the proteolytic processing of antimicrobial peptides, such as cathelicidins.¹²

Tomee *et al.*²⁰ showed that SLPI has activity (50% fungicidal activity) against human isolates of the pathogenic fungi *Aspergillus fumigatus* and *Candida albicans*. They also found partial inhibition of fungal protease activity by recombinant SLPI (rSLPI), a putative virulence factor of *A. fumigatus*, and subsequent inhibition of the inductive proinflammatory cytokine response in cultured human airway epithelial cell lines. In a recent study showed that the increase of salivary SLPI levels (to > 2.1 μ g/ml) along with other factors, such as low levels of CD4, antiretroviral monotherapy, and smoking, is a key predictor of oral candidiasis in human immunodeficiency virus type 1 (HIV-1)-infected persons. A possible biological explanation for this association is that SLPI is up-regulated in response to the infection in order to kill the pathogen and resolve the disease. An individual threshold limit to SLPI production

and secretion may be reached. Under this condition, the oral defenses are overwhelmed by the fungal insult and clinical disease ensues. In this scenario, an increase in salivary SLPI is associated with greater odds of having oral candidiasis and thus may be a marker of oral fungal disease. SLPI may also serve as an indicator of previous oropharyngeal candidiasis infection in the latter.

As with the antibacterial-bactericidal activity, the antifungal activity was mainly localized in the NH₂-terminal domain. It is believed that killing of fungus protects the epithelia from the fungal proteases. Probably the antibacterial and antifungal activities are related to the cationic nature of SLPI.²⁰ Given its antimicrobial activity, SLPI may provide a valuable therapeutic option in the future treatment or prevention of infectious diseases.

Wound healing and repair

Just after a surgical incision, a number of epithelial cells and connective tissue cells die and the basement membrane is disrupted. This clean and uninfected injury is enough to target an inflammatory response that will be absolutely necessary for the wound healing. Immediately after the incision, the wounds covered with clotted blood containing fibrin and blood cells. This fibrin clots receives within 24 hours an amount of neutrophils, attracted by inflammatory factors locally released. At this time, we also have mitotic activity of the basal layer of the epidermis. By the day 3, macrophages are the most common cells in the tissue, instead of neutrophils. The main feature at this moment is the granulation tissue, that consists of fibroblasts and new capillary with amorphous substance all around. By the 5th day, granulation tissue and neovascularization are maximal. Collagen fibrils are present and begin to bridge the incision, following the epithelial migration. After 1 week there is still connective tissue proliferation, but inflammatory features have virtually disappeared. At the end of the first month, the scar is completed within an intact epithelial layer, covering a new cellular connective tissue net, devoid of inflammation.²¹

In some instances, the wound (not surgical ones) has a large loss of cells and tissues, which makes the normal healing event impossible. In this case, we have the healing by second intention. This is characterized by a more complicated process with much more inflammation and granulation tissue. The original architecture is never attained and the main feature of the phenomenon is called wound contraction. The wound contraction is caused, at least in part, by the presence of myofibroblasts $\frac{3}{4}$ altered fibroblasts that have ultrastructural characteristics of smooth muscle cells.

As noted, the disposition of connective tissue matrix, specially collagen, its remodeling into a scar and the acquisition of wound strength are the ultimate effects of the repair.

The wound healing process is influenced by many systemic and local host factors. Nutrition state of the patient is very important. Protein deficiency and particularly

ascorbic acid deficiency inhibits collagen synthesis and impairs healing. Glucocorticoids therapy, by its anti-inflammatory aspects, retards healing. Patient's age is also an systemic factor that plays a role. Local infections are important causes of complicating and delaying healing process. Hemorrhagic factors, such as ischemia, play a role and foreign bodies, such as sutures and/or other fragments constitute impediments to healing.

The healing process may occur abnormally. There are many aberrations of growth, but the most common is called keloid. Keloid is a tumoral scar resulted from accumulation of excessive amounts of collagen. The reasons for keloid formation still remain unknown, but is known that it's more common in afro-caribbeans.

The response to injury is a phylogenetically primitive, yet essential innate host immune response for restoration of tissue integrity. Tissue disruption in higher vertebrates, unlike lower vertebrates, results not in tissue regeneration, but in a rapid repair process leading to a fibrotic scar. Wound healing, whether initiated by trauma, microbes or foreign materials, proceeds via an overlapping pattern of events including coagulation, inflammation, epithelialization, formation of granulation tissue, matrix and tissue remodeling. The process of repair is mediated in large part by interacting molecular signals, primarily cytokines, that motivate and orchestrate the manifold cellular activities which underscore inflammation and healing.

Clearance of debris, foreign agents, and/or infectious organisms promotes resolution of inflammation, apoptosis, and the ensuing repair response that encompasses overlapping events involved in granulation tissue, angiogenesis, and re-epithelialization. Within hours, epithelial cells begin to proliferate, migrate and cover the exposed area to restore the functional integrity of the tissue. Re-epithelialization is critical to optimal wound healing not only because of reformation of a cutaneous barrier, but because of its role in wound contraction. Immature keratinocytes produce matrix metalloproteases (MMPs) and plasmin to dissociate from the basement membrane and facilitate their migration across the open wound bed in response to chemoattractants. The migration of epithelial cells occurs independently of proliferation, and depends upon a number of possible processes including growth factors, loss of contact with adjacent cells, and guidance by active contact. TGF- β 1 stimulates migration of keratinocytes *in vitro*,^{6,19} possibly by integrin regulation and/or provisional matrix deposition.²⁰ Behind the motile epidermal cells, basal cell keratinocyte proliferation is mediated by the local release of growth factors, with a parallel up-regulation of growth factor receptors including TNF- α , heparin-binding epidermal growth factor (EGF) and keratinocyte growth factor (KGF or FGF-7).²¹⁻²³ Such growth factors are released not only by keratinocytes themselves, acting in an autocrine fashion, but also by mesenchymal cells and macrophages, as paracrine mediators.^{24,25} Numerous animal models in which cytokine genes have been deleted or over-expressed have provided

further evidence that such factors are involved in the process of epithelialization.²³ TGF- β 1, and TGF- β 2 are potent inhibitors of keratinocyte proliferation, with the Smad3 pathway implicated as the negative modulator. Since epithelialization is significantly accelerated in mice null for the Smad3 gene, with unchecked keratinocyte proliferation, but impaired migration in response to TGF- β 1, the implication is that the early proliferative event is critical to normal epithelialization.⁶ Once contact is established with opposing keratinocytes, mitosis and migration stop, and in the skin, the cells differentiate into a stratified squamous epithelium above a newly generated basement membrane. Other factors secreted by keratinocytes may exert paracrine effects on dermal fibroblasts and macrophages. One such factor is a keratinocyte-derived non-glycosylated protein termed SLPI which inhibits elastase, mast cell chymase, NF- κ B and TGF- β 1 activation. In rodents, SLPI is a macrophage-derived cytokine with autocrine and paracrine activities, but production by human macrophages has not yet been demonstrated. In mice, an absence of this mediator of innate host defense (SLPI null) is associated with aberrant healing.¹²

DISCUSSION

Secretory leukocyte protease inhibitor exerts its antiprotease activity by means of its COOH-terminal domain (C-terminal domain), and the active center of which is formed by the Leu⁷²-Met⁷³ residues.¹ The NH₂-terminal domain (N-terminal domain) has no such properties, but it may aid in stabilizing the protease-antiprotease complex and may mediate the enhancement of the antiproteinase activity of SLPI by heparin. Heparin augments the effectiveness of SLPI as it induces a conformational change in the inhibitor.²³ In addition, SLPI increases glutathione levels, thereby reducing oxidant-mediated tissue injury, and prostaglandin E₂ and matrix metalloproteinases are reduced. Hiemstra¹² hypothesized that SLPI's cysteine residues are utilized for the glutathione synthesis.

SLPI inhibits the pro-inflammatory activity of bacterial products such as lipopolysaccharide and regulates the activity of inflammatory cells. This has been suggested to be due to inhibition of activation of the transcription factor nuclear factor- κ B (NF- κ B) by SLPI, as a result of inhibition of the proteolytic degradation of I κ B, the inhibitors of NF- κ B, in unstimulated cells, NF- κ B is retained in the cytoplasm in complex with I κ B proteins. Upon cellular activation, I κ B is degraded and NF- κ B is released, allowing it to move to the nucleus and influence gene expression. Therefore SLPI-mediated protection of I κ B from proteolytic degradation may inhibit NF- κ B activity and its ability to increase the expression of pro-inflammatory genes.²⁴

A role for SLPI in tissue repair was suggested by the observation that the epithelial expression of SLPI is increased upon cutaneous injury in humans. Whereas these observations suggest an association between tissue

repair and SLPI expression, In that study it was shown that the absence of SLPI resulted in delayed cutaneous wound healing, which was attributed to an increased and prolonged inflammatory response during the repair process and delayed matrix accumulation

SLPI stimulate the production of hepatocyte growth factor (HGF). HGF is a major cytokine product of mesenchymal cells and has been implicated in the regulation of mitogenesis, motogenesis and morphogenesis. In addition to regulating HGF production by fibroblast, SLPI also affect other function of these cells, such as their ability to contract collagen gels in vitro. Collagen gel contraction is thought to result from the ability of fibroblast to reorganize and compact collagen fibres, and the model is considered as in vitro model of wound healing and scar formation. Analysis of the ability of conditioned medium from cultured human oral epithelial cells to contract collagen gels in vitro led to the identification of SLPI as factors in this medium that inhibits fibroblast-mediated scar formation.

The oral epithelium also forms part of an intercommunicating network of immune system, in which signals are regularly exchanged in dynamic interactions. Oral epithelial cells produce a range of cytokines including interleukin-1 beta (IL-1 β), interleukin-6, tumor necrosis factor-alpha (TNF- α), granulocyte-macrophage colony stimulating factor (GM-CSF), transforming growth factor-beta (TGF- β) and their receptors and IL-8.²⁵

Three major function of SLPI can be delineated: inhibition of local elastase, controlling of leukocyte activation and reduction of TGF- β activation, leading to a reduced inflammatory response. Aside from suppressing elastolytic release of TGF- β complexed with elastin, however, SLPI appears to control TGF- β activity by regulating cellular activation.² The local induction of SLPI might be important to break the cycle of inflammation. However the mechanisms involved in the regulation of SLPI expression and release still remain to be elucidated. It has been shown that SLPI is up-regulated by pro-inflammatory stimuli including LPS, TNF α , IL-6 and IL-1 β , in vitro.²⁶ In conclusion SLPI has a multifaceted role in oral wound healing: indeed, SLPI confers local protection against microbial, fungal and SLPI is a pivotal endogenous factor necessary of optimal wound healing.

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