Secretory leukocyte protease inhibitor as an indicator of periodontal disease

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Dedicated to my parents, my wife and my kids
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ABSTRACT

Based on a very limited information available that measures Secretory Leukocyte Protease Inhibitor (SLPI) levels in relation to periodontal disease and oral secretions. SLPI exhibits antimicrobial activities, which may play a critical role in mucosal defenses. The purpose of this study is to investigate the relationship between SLPI concentrations and periodontal disease. A total of 36 subjects (17 females & 19 males) were selected from patients (n=18) who were treated for moderate to severe periodontitis and healthy staff members (n=18). SLPI level in saliva was determined with ELISA. At baseline, there were no differences in age or gender distribution between test and controls. Results showed that, the salivary concentration of SLPI did not differ between patients and control. Furthermore, there were no differences between males and females or between smokers and non-smokers. No relations detected between SLPI concentrations and bleeding and probing, number of pockets and age, respectively. In conclusion, this study does not support the concept of an involvement of SLPI in the pathogenesis of periodontitis. SLPI is not a good indicator of periodontal inflammation.

Key words: Secretory leukocyte protease inhibitor, periodontal disease, Saliva, ELISA,
INTRODUCTION

Phagocytic cells migrate from the peripheral circulation to the site of infection (Henson et al. 1988). These cells, especially granulocytes, contain enzymes that can degrade key plasma proteins and components of the extracellular matrix, thereby damaging host tissues (Janoff, 1985). For this reason, there has been significant interest in the distribution and properties of leukocytes protease inhibitors. These proteins are thought to be important in regulating the local activity of the proteases through rapidly forming strong complexes with the enzymes (Thompson RC et al. 1986). Proteinase inhibitors are thought to play an important role in the regulation of the extracellular action of proteinase, such as the serine proteinase human leukocyte elastase, that is released from stimulated neutrophils. Elastase can cause extensive tissue degradation and has been shown to be involved in several diseases (Hiemstra et al. 1996).

SLPI is composed of 2 cysteine – rich domain with a protease inhibitory site situated at leucine 72 in the carboxy-terminal domain (Gillian et al. 2000). SLPI is a 11.7 kD acid stable highly basic protein produced by epithelial cells lining mucosal surfaces (Franken et al. 1989). Originally isolated from human parotid gland fluids (Ohlsson et al. 1984 ) and it is present on average at 106.9 ng/ml in saliva (Baqui et al. 1999), the mucosal protein inactivates serine protease such as neutrophil elastase during inflammation .
Amino acid sequence of SLPI led to the prediction that the protein consists of two highly homologous domains of 53 and 54 amino acids. Two regions of the protein were identified as being likely sites of interactions with proteases (Thompson & Ohlsson, 1986).

One of these, present in the COOH-terminal domain of SLPI, was proposed to be the site interacting with chemotrypsin-like enzymes and elastase. The other region, present in the homologous position of the NH2-terminal domain, has been proposed as the site of interaction with trypsin (Stephen et al. 1990).

The affinity of the inhibitor for leukocyte elastase is very high, suggesting a functional role for the protein in preventing elastase-mediated damage to oral and possibly other mucosal tissues (Thompson & Ohlsson, 1986).

It was eventually recognized to have other effects, such as antagonizing lipopolysaccharide induced production of tumor necrosis factor - α (TNF-α) in stimulated phagocytic cells (Jin et al. 1997) and interfering with the entry of HIV into susceptible cell lines (McNeely et al. 1995).

Recent studies have identified novel inhibitory activities for SLPI against commensal fungi (e.g., C albicans) (Klassen & Kramps, 1985), bacteria (e.g. E. coli and Staphylococcus aureus) (Hiemstra et al. 1996) and Viruses (e.g. HIV (Jin et al., 1997) and Influenza virus) (McNeely et al. 1995) as well as a role in coetaneous wound healing (Gillian et al. 2000).
Based on these findings, SLPI is thought to contribute to the host mucosal defense of oral (Ohlsson et al. 1984), nasopharyngeal (Westin et al. 1994), genital (Casslen et al., 1981) and respiratory (Dijkman et al., 1986) tissues. SLPI accounts for 80 - 90 % of the human neutrophil elastase – inhibitory activity present in sputum (Baqui et al. 1999).

Mucosal transmission accounts for most infections with HIV-1. Unlike other mucosal sites, the oral cavity is an infrequent route of HIV-1 transmission (Shugars & Wahl. 1998). Infectious viruses is rarely isolated from saliva, confirming the virtual lack of oral transmission reported by numerous epidemiological studies (Diane & Shugars, 1999), which added to the functions of SLPI.

In addition to its primary functions relevant to innate host defense, including antimicrobial and anti-inflammatory activity, the control of intracellular enzyme synthesis, and the suppression of monocyte matrix metalloproteinase production and activity. SLPI also antagonizes (LPS) - induced pro-inflammatory mediator synthesis by monocytes and macrophages. When viewed in the context of a chronic non-healing wound, in which inflammation and bacterial infection contribute to delayed healing, SLPI is a potential candidate for enhancing the healing response, however, the major function of SLPI as a local anti-elastase agent in the most intriguing (Gillian et al. 2000).
THE AIM

Our hypothesis is that oral SLPI concentration is altered in the presence of periodontitis. So in this study we will compare SLPI concentrations in unstimulated salivary secretion of healthy and periodontitis subjects.
MATERIALS AND METHODS

The study was approved by the ethics committee at Huddinge University Hospital, Sweden.

A total of 36 subjects (17 females & 19 males) were selected from patients who were treated for moderate to severe periodontitis in the periodontal clinic, and from the staff, at the Institute of Odontology, Karolinska Institutet, Sweden. Informed consent was obtained from each subject prior to participation.

Criteria for exclusion included age less than 25 years, people with a history of antibiotic intake during the last 3 months, medically compromised patients (AIDS, kidney or liver transplanted), and other systemic infections which could need additional medications.

Subjects

 Totally were 36 subjects included in the study. 18 were allocated as a test group (all of them were treated for periodontitis). The other 18 subjects allocated as a control group were selected from the staffs that were sex- and age- matched those of the test. Details about the study population can be found in Table 1.
Table 1. Study population; age, gender, smoking habits and clinical data.

<table>
<thead>
<tr>
<th>Category</th>
<th>Age (year)</th>
<th>Gender</th>
<th>Smoking</th>
<th>No. of teeth</th>
<th>B o P (%)</th>
<th>No. of pockets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>M/F</td>
<td>S/NS</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Patients n=18</td>
<td>55.3 (9.2)</td>
<td>10/8</td>
<td>9/18</td>
<td>23.4 (5.4)</td>
<td>30.8 (16.8)</td>
<td>27.5 (20.9)</td>
</tr>
<tr>
<td>Controls n=18</td>
<td>51.1 (10.9)</td>
<td>9/9</td>
<td>1/17</td>
<td>28.3 (2.1)</td>
<td>18.8 (12.5)</td>
<td>2.60(2.3)</td>
</tr>
</tbody>
</table>

Collection of saliva

Whole human saliva (~5ml, unstimulated) was collected from each periodontitis and control patients by tilting the head forward and dribbling saliva from the lower lip into a 50-ml graduated centrifuge tube. After 5 minutes, the subject was asked to expectorate any remaining saliva. The saliva sample stored immediately in a freezer (-20°C) until used for SLPI estimation. The samples were centrifuged at 11,000 g for 5 minutes.

SLPI assay

SLPI levels in saliva was determined with a commercial enzyme-linked
Immunosorbant assay (ELISA). Secretory leukocyte protease inhibitor levels were determined using an SLPI-ELISA system (R & D Systems,
Minneapolis, MN, USA). Murine monoclonal antibody against human SLPI was coated onto 96-well plates.

Polyclonal antibody against human SLPI conjugated to horseradish peroxidase was used as a secondary antibody. Recombinant human SLPI was used as a standard for construction, calibration curves. Plates were read using a Millenia Kinetic Analyser, Diagnostic Product Corp. (Los Angeles, CA, USA) at 450 nm. Amounts of SLPI were expressed in ng/ml. The ELISA procedure employed a quantitative solid-phase sandwich enzyme immunoassay technique wherein a monoclonal antibody specific for SLPI was used to coat the micro-titer plate provided in the kit. The saliva will be diluted 50-fold with the solution provided in the kit. Also, SLPI assay experiments will be repeated with further dilution of the samples when the values of unknown samples are above the highest of the standard value.

Statistics:

The data were analyzed statistically and presented as medians and interquartile range. Mann Whitney-U test was used to test the differences between the groups and Spearman rank test to test the significance of the correlations. The Statistica 6.1 computer program was used to analyze the data.
RESULTS

At baseline, there were no differences in age or gender distribution between the two groups (Table 1). The mean age was 55.3 years and 51.1 years, for patients and controls, respectively. 10 out of 18 patients were male, while among the controls, out of 18, 9 subjects were males.

The salivary concentration of SLPI did not differ between patients and control as presented in (Fig.1).

Fig. 1. Box plot showing distribution of SLPI concentrations among patients with periodontitis and healthy controls. Boxes indicate 25-75 % and vertical bars 10-90%.
Furthermore, there were no differences between males and females (Fig 2) or between smokers and non-smokers (Fig 3).

Fig. 2. Box plot showing distribution of SLPI concentrations between male and females. Boxes indicate 25-75 % and vertical bars 10-90%.

Fig. 3. Box plot showing distribution of SLPI concentrations between smokers and non-smokers. Boxes indicate 25-75 % and vertical bars 10-90%.
The association between SLPI concentration in saliva and bleeding and probing, number of pockets and age respectively, did not show differences (Fig. 4-6).

\[ r^2 = 0.06, p=0.22 \]

**Fig 4.** Correlation between SLPI concentrations in saliva and bleeding on probing. \( n=36 \) subjects

\[ r^2 = 0.06, p=0.15 \]

**Fig 5.** Correlation between SLPI concentrations in saliva and number of pockets. \( n=36 \) subjects.
$r^2 = 0.04, p=0.27$

Fig 6. Correlation between SLPI concentrations in saliva and age. n=36 subjects.
DISCUSSION

The findings from the present study were not able to show a difference in salivary SLPI concentrations between patients with periodontitis and periodontally healthy controls. There were no associations have been detected between clinical gingival inflammation, registered as bleeding on probing, and SLPI. A reason for this finding could be that most of the SLPI in saliva originates from the salivary gland and not from gingival crevicular fluid (GCF). Furthermore, we could not make any correlation between SLPI concentration with pocket depth and age. There is a trend that the levels of SLPI reduced with age which is in agreement with previous study (Shugars et al. 2001). They reported that SLPI diminished with advancement of age among 45 non-hospitalized dentate adults aged 79-89 years compared to young adults aged 21-51 year. In our study, we compared the younger (≤45 years) to the olders (≥65 years), and we expected that, if the number of involved subjects increased to be more than 20 subjects in each group, we would be able to find some difference. The results also showed no relationship between number of teeth and SLPI concentrations. Moreover, no difference found between smokers and non-smokers in relation to the salivary SLPI concentrations. One explanation of this is that most of smokers are of old age.
The use of saliva as a source of components that may identify subjects at risk of developing destructive periodontitis, or provide markers of disease potential or activity has been reviewed. Saliva may prove to be useful as source of indicator of current disease activity or as a means of assessing response to treatment (Wilton et al. 1989). Clinical indicators may reflect only past inflammation, and cannot distinguish between active and inactive sites. Secretory leukocyte protease inhibitor (SLPI), considered as a major human elastase inhibitor (Kramps et al. 1988; Sallenave et al. 1997). Elastase can cause extensive tissue degradation and has been shown to be involved in several diseases (Stockly, 1987).

Based on a very limited information available that measures SLPI levels in relation to periodontal disease and oral secretions. In a study of Minami et al 2003 he measured SLPI in GCF in twenty-one patients who were treated for moderate to severe periodontitis. GCF samples were collected from forty-one sites before scaling and root planing, 2 and 4 weeks after scaling and root planing. SLPI increased significantly at 2 weeks in sites that bled on probing BoP(+), while SLPI did not significantly differ at both time points in BoP(-) sites and at 4 weeks in BoP(+) sites.

To our knowledge, there is no previous report that measured SLPI levels in Saliva of periodontitis patients. However, the present study is the first evaluation of salivary SLPI concentration in healthy individuals and in those with periodontal disease.
This study has certain limitations. Sample size was small and there were more smokers in the patient group. However, a power calculation indicates that even if we had increased the study population to 100 subjects, the difference between patients and controls had still not been significant. More work is needed to study SLPI levels in saliva, which may identify the possible risk of periodontitis. It would probably be necessary to discriminate between SLPI originating from the salivary glands and that from the gingival crevice.

In conclusion, this study does not support the concept of an involvement of SLPI in the pathogenesis of periodontitis. SLPI is not a good indicator of periodontal inflammation.
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REFERENCES


