The Role of Loricrin in Severe (Formerly Aggressive) Periodontal Disease

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Abstract

Objective: Severe grade C periodontal disease (formerly known as Aggressive periodontitis; AgP) comprises a group of rapidly progressive forms of periodontitis generally characterized by an early age of clinical manifestation and a distinct tendency to aggregate in families. Research has suggested multiple aetiologies for AgP, but no common mechanism. The overall goal of our study is to determine if down regulation of loricrin leads to impairment of epithelial barrier function and increased levels of inflammation and bone destruction in the periodontium in response to bacteria involved in AgP.

Methods: Gingival samples were collected from periodontally healthy patients and AgP patients undergoing routine periodontal surgeries in which the tissue is normally discarded. ELISA was used for protein detection. This study was approved by the research ethics board of the University of Alberta (Pro00062112).

Results: A total of 12 samples from AgP patients and 11 samples from healthy control patients were collected. An ELISA was performed to compare the amount of loricrin protein in the samples. The average concentration of loricrin was significantly higher in the healthy group (9.240±1.572ng/ml) than in the AgP group (2.813±0.8583ng/ml) ($p=0.0008$, MannWhitney test).

Conclusions: These results suggest that patients with AgP have less loricrin protein expression, consistent with gene expression studies. Based on these results we now hypothesize that decreased loricrin protein may result in a compromised oral barrier by disrupting normal epithelial differentiation, and this may explain why biofilm bacteria cause such a dramatic inflammatory response in AgP patients.

Introduction

Aggressive periodontal disease (AgP), now termed Grade C periodontitis, involves rapid destruction of the periodontium often leading to early tooth loss (Figure 1, Figure 2)[1,2]. However, the accelerated rate of destruction observed in this disease remains unexplained. An important clinical finding in AgP is the presence of minimal plaque biofilm, which does not correlate with the degree of damage and intensity of the inflammation.[3,4] Although it is doubtless that bacteria are a major player in the disease, it is unclear why a certain subset of patients has an exaggerated response. Patients require early diagnosis and frequent professional appointments for scaling and oral hygiene instruction in order to maintain their teeth. Given the minimal plaque present, it appears as if the host is responding to a much greater peril than present. An alternative hypothesis, however, is that the host response is actually appropriate for the threat, but there is some other factor that increases the threat. This "factor" may be a compromised barrier. When the epithelial barrier of the sulcus is healthy and functioning normally, pathogenic bacteria are excluded. However, if for some reason the epithelial barrier is compromised, the host will be exposed to pathogenic bacteria, leading to an inflammatory response.

The cornified epithelium (CE) is the outermost layer of the skin and oral mucosa, optimized for barrier function.[5] The CE proteins responsible for this barrier function include: involucrin, cystatin A, loricrin, small proline-rich proteins, elafin, proteins of the S100 family, profilaggrin and some desmosomal components.[6] Loricrin is a 26 kilodalton (kDa) insoluble protein that constitutes approximately 70-85% (volume) of the CE in differentiated corneocytes.[7-11] The inter- and intra-protein crosslinks formed by loricrin are highly resistant to proteolysis and stabilize and strengthen the CE.[12] Therefore, if loricrin expression is downregulated, it is reasonable to suspect a potential impact on the barrier function of the CE (Figure 3).

Barrier function plays an important role in inflammatory skin diseases. Psoriasis and atopic dermatitis are two such diseases in which patients present with loricrin downregulation.[13,14] Psoriasin is

upregulated in both psoriasis and atopic dermatitis and has been shown to inhibit epidermal differentiation by reducing the expression of key proteins like loricrin.[16] The lack of loricrin and other epidermal proteins may compromise the epithelial barrier and consequently, lead to the host's chronic inflammatory response to skin bacteria. Interestingly, psoriasis has even been recently associated with chronic periodontal disease.[17-21] Loricrin specifically has not been thoroughly investigated with regards to periodontal diseases, however, the parallel observed between barrier dysfunction and bacteria in other diseases supports the potential for an association.[22]

Since loricrin is the most abundant protein in the CE layer, it follows that its expression in gingival epithelium may be important to the functionality of the CE. In two unbiased studies of genes regulated in AgP, loricrin was found to be significantly downregulated. One study showed loricrin was decreased 7-fold in AgP patients compared to healthy controls and a second study showed loricrin was decreased by 25% in AgP patients compared to chronic periodontal disease patients.[23,24]

Statement of purpose

Based on the current literature, our overall hypothesis is that loricrin downregulation may result in an impaired oral epithelial barrier which could then lead to the increased inflammatory response and profound damage observed in AgP-susceptible patients. The primary objective of the present study is to compare loricrin protein expression in healthy control and AgP patients.

Methods

Sample Collection

Healthy and AgP patients were identified and diagnosed by a periodontist. Patients who met the criteria provided informed consent prior to entering the study. Healthy patients were diagnosed as periodontally healthy, showed periodontal pockets no greater than 4mm and no evidence of radiographic bone loss. Healthy patient tissues were collected during crown lengthening procedures. AgP patients were diagnosed during their active state and their tissues were collected during periodontal surgery. Tissues from patients were collected and placed in RNAlater (Qiagen, Cat No.16706) (which also allows for protein isolation) and stored at -80^{o}C. Ethical approval was obtained from the University of Alberta Ethics Board (Pro00062112).

Sample Preparation

Human tissue samples were prepared according to the Cloud Clone Corp. ELISA protocol for human loricrin (Cloud Clone Corp, SEC568Hu). Samples were weighed to normalize for the amount of tissue, and proteins extracted by mincing and sonication in lysis buffer included in the kit. Samples were centrifuged at 10,000 g for 5 minutes for removal of debris and stored at -20^{o}C. The final concentration of each sample was adjusted to 50mg/ml.

ELISA Protocol

The contents of the kit and samples were brought to room temperature. A standard curve was created by serial dilution of loricrin provided, and the assay conducted as per the manufacturer's instructions. 100 ul of controls, standards and sample were assayed in duplicate. Data were collected using a Synergy H1 Hybrid Multi-Mode spectrophotometer. Loricrin concentration was assessed in samples on the basis of the standard curve. Statistical analysis was performed using GraphPad Prism Software. The data were tested for normal distribution using the Shapiro-Wilk test. Because the data were found to not be normally distributed, a Mann Whitney test, was performed to determine differences.

Results

A total of 11 samples were collected from healthy patients while 12 samples were collected from patients diagnosed with AgP. AgP samples were from patients under 35 years of age. Healthy patient samples were from individuals whose age averaged 51.5 $\pm$ 7.60 years. As shown in Figure 4, the mean $\pm$ S.E. was 9.874 $\pm$ 1.665 for healthy patients and 2.813 $\pm$ 0.8583 for AgP patients. This is approximately a 61% reduction. The results were significant ($p<0.001$, Mann Whitney test). The Mann Whitney test, a non-parametric test, was indicated as the data were not normally distributed, as assessed by the Shapiro-Wilk normality test.

Discussion

An intriguing clinical characteristic of AgP patients in contrast to chronic periodontitis patients is the presence of only minimal plaque biofilm.[3,4] AgP patients may present with little to no calculus and bacterial load often does not correlate with the intensity of the inflammation and degree of bone loss. This exaggerated response is as if the host is responding to a much greater challenge than what is actually present. The hypothesis of this study is that the host response may instead be appropriate for the threat if the host epithelial barrier is compromised.

Loricrin, the most abundant protein in the CE is a protein of interest in AgP for the following reasons: Firstly, loricrin has been shown to play a critical role in atopic dermatitis.[25] Patients with this inflammatory skin disease have decreased loricrin protein in their skin, and as a result, are more susceptible to pathogen induced inflammation.[25] Secondly, loricrin has been previously observed to be downregulated at the mRNA level in patients with AgP. The primary objective of the present study was to compare loricrin protein expression in healthy control and AgP patients.

Our ELISA data showed a significant reduction in loricrin protein oral tissue samples from AgP patients compared with healthy controls. This is noteworthy because, to our knowledge, this is the first study to compare loricrin protein expression in AgP and healthy patients. A previous study evaluated gene

expression in both healthy and AgP patients (Guzeldemir 2016). The study compared gene expression by microarray following RNA isolation from gingival biopsies from 23 AgP patients and 25 healthy individuals.[23] The results of this study showed that loricrin gene expression was downregulated almost 7-fold in patients with AgP.[23] Thus, these findings at the mRNA level are validated at the protein level by our present study. These data provide support for the hypothesis that AgP patients have significantly less loricrin protein expression than healthy patients, and this could impair epithelial barrier function.

Limitations

Due to limited resources, sample size of the AgP samples are a limitation to the study. Furthermore, tissue samples were also retrieved from one spot in the patient's mouth which may lead to sample bias and the ages of the control and AgP patients were not matched.

Conclusion

Loricrin, the most abundant CE protein, plays an important role in maintaining barrier function. A validated human loricrin-specific ELISA showed that loricrin protein expression was significantly reduced in patients with AgP (or Grade C Periodontitis). This reduction in loricrin may compromise CE function, which in turn may underlie the exaggerated response to plaque in these patients. Although there are other factors contributing to this disease, decreased loricrin protein expression may be considered a risk factor for AgP/Grade C Periodontitis.

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Figures

Figure 1: A 23 year old AgP patient with significant bone loss and early tooth loss.

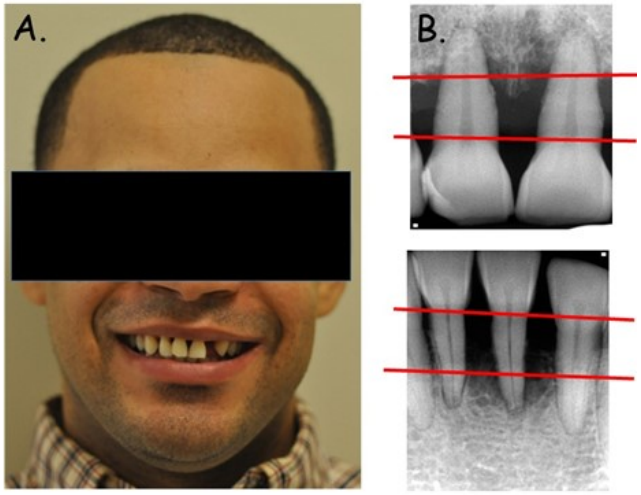

Figure 2: Radiographic view of localized AgP in a 13 year old patient.

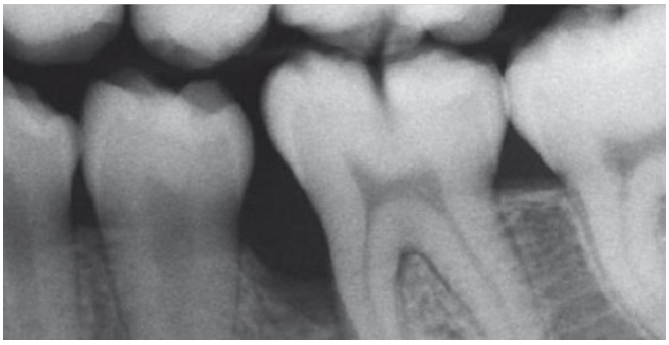

Figure 3: A visual comparison of a systemically healthy patient with bacteria challenge. The left represents a patient with normal expression of loricrin. The right represents an otherwise systemically healthy patient with lowered loricrin expression.

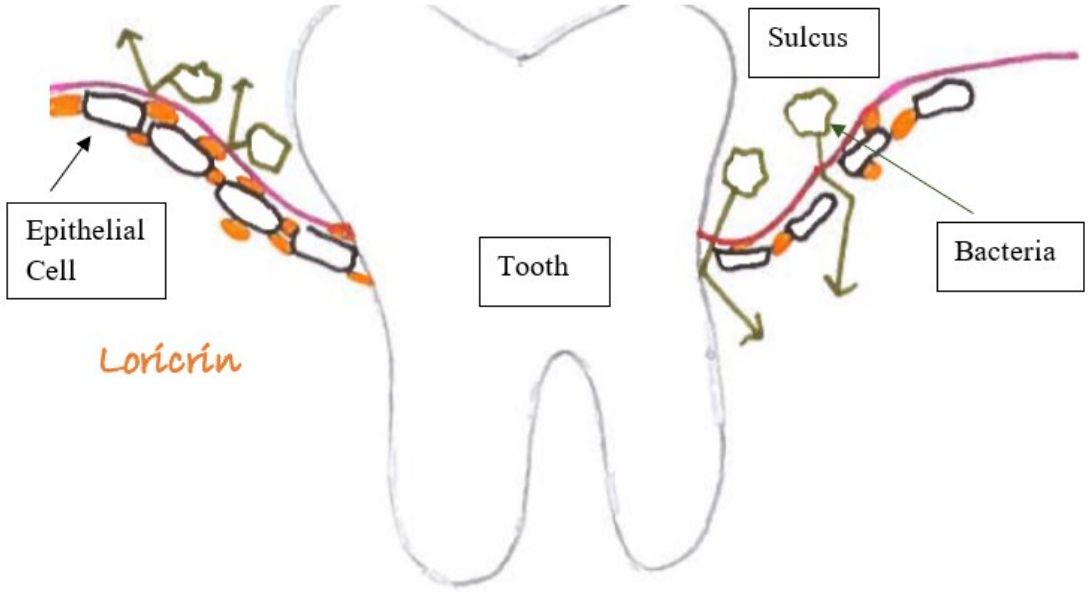

Figure 4: ELISA of 11 healthy and 12 AgP gingival tissue samples.