Up-regulation of Surfactant Protein A in Chronic Sialadenitis

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Objectives: Salivary secretions play a critical role in maintaining the health of the oral cavity, which is the first gate of entry to the airways and thus is exposed to a variety of environmental insults. Surfactant protein A (SP-A) is a member of the collectin family and plays an important role in first-line airway defense. The objectives of this study were to examine the expression of SP-A messenger RNA and protein in human salivary glands and to investigate its up-regulation during inflammatory conditions.

Design: Reverse transcription–polymerase chain reaction was performed on salivary gland tissues from patients and a control group. The expression levels of SP-A to GAPDH (glyceraldehyde-3-phosphate dehydrogenase) transcripts were semiquantified by densitometry. We also characterized the cellular localizations of SP-A protein immunohistochemically.

Setting: Tertiary academic institution.

Patients: Ten patients with chronic sialadenitis and 10 patients with healthy salivary glands.

Results: Surfactant protein A messenger RNA and protein were detected in glands of patients who were healthy and in those with chronic sialadenitis. The expression levels of SP-A messenger RNA in the salivary glands of patients with chronic sialadenitis was significantly increased compared with those in healthy salivary glands. Immunohistochemical staining revealed SP-A immunoreactivity in the ductal epithelia of healthy salivary glands and in the salivary glands of those with chronic sialadenitis, and stronger immunoreactivity was observed in those with chronic sialadenitis tissues.

Conclusions: Surfactant protein A is present in the salivary gland epithelium and is up-regulated in individuals with chronic sialadenitis. These results suggest that salivary gland SP-A may play an important role in the innate host defense of human salivary glands.


The oral cavity and the salivary gland ducts are open to the oral environment and thus are exposed to multifarious biological, chemical, and mechanical insults. Oral mucosal and salivary glandular defenses range from simple mechanical rinsing by salivary flow to complex mechanisms of host defense and adaptive immunity.

The hydrophilic surfactant protein A (SP-A) is a major protein component of lung surfactant that belongs to the collectin family. This protein is secreted by airway epithelial cells and is present in airway lining fluid, where it contacts and binds to the surface structures of a variety of microorganisms soon after they have been inhaled. Also, SP-A regulates innate immune cell functions and therefore supports the first-line defense against pathogenic microorganisms.1-3 Definitive proof of the contribution made by this protein to airway host defense was provided by studies4,5 on SP-A knockout mice. The SP-A knockout mice are more susceptible than wild-type mice to infections with bacterial and viral pathogens, and SP-A deficiency was associated with enhanced inflammation and synthesis of proinflammatory cytokines. In addition to its role in innate immunity, SP-A also participates in adaptive immune reactions.6

Recently, Madsen et al7 reported that SP-A messenger RNA (mRNA) is expressed in the healthy human salivary gland, an extrapulmonary tissue. To the best of our knowledge, there have been few reports about the association between inflammation and SP-A expression in extrapulmonary tissues. We hypothesized that the expression of SP-A would differ under inflammatory conditions compared with the healthy state in the human salivary gland.

The present study describes the differential expression of SP-A in the salivary gland in healthy and chronically inflamed states as determined by reverse transcription–polymerase chain reaction (RT-PCR) and immunohistochemical analysis.
Figure 1. Reverse transcription–polymerase chain reaction for surfactant protein A (SP-A) messenger RNA (mRNA) in the salivary glands of healthy controls and of patients with chronic sialadenitis. The SP-A mRNA was expressed in both healthy salivary gland tissues and in the tissues of patients with chronic sialadenitis. The expression level of SP-A mRNA was significantly increased in tissue from patients with chronic sialadenitis compared with tissue from healthy controls. The plus sign indicates a positive control; minus sign, a negative control; bp, base pair; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

**TISSUE SAMPLES**

Tissue samples were obtained from surgical biopsies performed at the Department of Otorhinolaryngology–Head and Neck Surgery, Korea University College of Medicine, Seoul. Salivary gland tissues were collected from the excised parotid glands of 10 patients with sialadenitis and from the distant healthy salivary tissues of 10 patients with benign parotid gland tumors. One portion of tissue was immediately frozen in liquid nitrogen and stored at −70°C for subsequent RNA studies. For RT-PCR–positive control of SP-A, samples of lung epithelium were prepared in the same manner. Another portion of the salivary tissue was fixed with 4% paraformaldehyde in 0.1M phosphate-buffered saline (pH 7.4) overnight at 4°C and then embedded in paraffin for immunohistochemical staining. Informed consent was provided by all study subjects, and tissue procurement procedures were approved by the institutional review board of Korea University.

**EXTRACTION OF RNA**

Tissues were homogenized in 1 mL of Trizol reagent (Gibco BRL, Tucson, Ariz), and RNA was extracted according to the manufacturer’s instructions. The RNA samples were air dried and resuspended in water treated with diethyl pyrocarbonate, and either kept on ice for immediate use or stored at −70°C. Aliquots of RNA were treated with RNase-free RQI Dnase (Promega, Madison, Wis) according to the manufacturer’s instructions. The RNA concentrations were determined spectrophotometrically, and RNA integrity was checked by electrophoresis in agarose gels containing formaldehyde.

**RT–PCR REACTION**

The total RNA from each sample was reverse transcribed in 20 µL of reaction mixture containing 2.5 U of Maloney murine leukemia virus reverse transcriptase (Gibco BRL) and 50 pmol of random hexanucleotides at 42°C for 60 minutes. Amplification of the complementary DNA so formed was carried out at 35 cycles at 94°C for 45 seconds, 55°C for 30 seconds, and 72°C for 1 minute, followed by a final extension cycle at 72°C for 7 minutes. Sense primer for SP-A was 5’-GGA AGC CCT GGT ATC CCC GG-G3; antisense primer, 5’-TAA TGG TAT CAA AGT TGA CTG-3’. The sense primer 5’-GGT GAT ATT GTC ATG GCC ATC AAC GAC C-C3 and the antisense primer 5’-GCC CCA GCC TGC ATG GTG GTG-3’ for GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were run simultaneously as an internal control. The RT-PCR products were analyzed by gel electrophoresis. The predicted size, restriction digestion, and DNA sequencing verified specificity of the 372-base pair RT-PCR product for SP-A. To establish the specificity of responses, negative controls were used in which input RNA was omitted or in which RNA was used but reverse transcriptase was omitted. As a positive control, mRNA was extracted from lung tissues known to express SP-A. To ensure RNA quality, all preparations were subjected to analysis for GAPDH expression. To semiquantitatively analyze RT-PCR results, we scanned gel images and measured the intensity of each PCR product using NIH Image software (National Institutes of Health, Bethesda, Md). The relative intensities of SP-A bands on gel images are expressed as SP-A/GAPDH ratios.

**IMMUNOHISTOCHEMICAL STAINING**

Paraffin blocks were sectioned in 5-µm thicknesses. Sections were deparaffinized with xylene, and then serial rehydration with 100% and 75% alcohol was performed. Paraffin sections of parafformaldehyde-fixed salivary tissues were treated with 3% hydrogen peroxide/methanol to block endogenous peroxidase and then incubated with diluted (1:100) polyclonal goat antihuman SP-A antibody (Santa Cruz Biotechnology Inc, Santa Cruz, Calif) overnight at 4°C in a humidified chamber. Immunoreactive SP-A was visualized using a Vectastatin Elite ABC Kit (Vector Laboratories Inc, Burlingame, Calif). Nonimmune serum IgG or phosphate-buffered saline, rather than the primary antibody, was used as a negative control. The slides were counterstained with Mayer hematoxylin and mounted.

**STATISTICAL ANALYSIS**

Data are expressed as mean±SEM (Figure 1). Comparisons of quantitative data between the 2 groups were performed using the Mann-Whitney U test. Differences were considered significant for P values smaller than .05.

**RESULTS**

The RT-PCR products of the salivary tissues had the size (372 base pairs) that was expected from the selected primers. The same-sized products were expressed in the positive control. Surfactant protein A mRNA was detected in both healthy tissue and that of patients with sialadenitis (Figure 1). Surfactant protein A mRNA was amplified weakly in healthy salivary glands, whereas inflamed sali-
vary glands showed strong SP-A mRNA expression. The level of SP-A mRNA expression was significantly different in individuals with chronic sialadenitis and healthy salivary glands ($P<.05$) (Figure 2).

**IMMUNOHISTOCHEMICAL STAINING FOR SP-A PROTEIN**

Surfactant protein A expression was found to be localized to the ductal cells in individuals with chronic sialadenitis and those with healthy salivary glands; acinar cells were uniformly negative for SP-A immunostaining (Figure 3). Ductal cells in patients with chronic sialadenitis immunostained more positively than cells in those with healthy salivary glands. There is no immunoreactivity for SP-A in acinus. No specific localization was found using the negative control, which confirmed the specificity of the SP-A antibody.

In the present study, we demonstrated the status of SP-A mRNA and protein expression in healthy and in chronically inflamed salivary glands. The RT-PCR showed that SP-A mRNA expression levels are elevated in chronic sialadenitis. It also demonstrated not only that the SP-A gene is expressed constitutively but also that its expression is up-regulated during inflammation. Immunohistochemical staining showed that SP-A protein is localized predominantly in the ductal epithelial cells of the salivary gland.

Surfactant protein A is known to play a role in the host defense against pathogens. It interacts with the cell surfaces of microorganisms and modulates immune cell functions. In this manner, SP-A augments the phagocytosis of bacteria and viruses by macrophages, monocytes, and neutrophils in a concentration-dependent manner and also enhances neutrophil migration. Moreover, SP-A has been shown to provide a link between innate and adaptive immune responses by modulating the differentiation and chemotaxis of dendritic cells. It has also been shown that SP-A binds to and neutralizes viruses. In addition, SP-A promotes the attachment of mycobacterium tuberculosis to alveolar macrophages in individuals with human immunodeficiency virus infection. Furthermore, human SP-A has been reported to bind certain types of fungi.

Locally produced factors that modulate the host defense against pathogens represent an early and critical aspect of host defense. Although SP-A was first identified as a component of the pulmonary surfactant complex, a growing body of evidence indicates that it is also expressed in other organs. Extrapulmonary expression of SP-A has been found in otitis media effusion products, in the epithelial cells lining the gastrointestinal tract and mammary glands, in the maxillary sinus, middle ear, and eustachian tube; in mesentery cells, in the prostate gland and in the thymus; and in the synovium. Luo et al. found immunoreactivity to SP-A in the salivary gland duct. The demonstration of SP-A protein in the salivary gland epithelium in the present study contrasts with observations from Madsen et al., who found expression of SP-A in healthy and inflamed salivary glands. The RT-PCR showed that SP-A mRNA expression levels are elevated in chronic sialadenitis. It also demonstrated not only that the SP-A gene is expressed constitutively but also that its expression is up-regulated during inflammation. Immunohistochemical staining showed that SP-A protein is localized predominantly in the ductal epithelial cells of the salivary gland.
mRNA but detected no SP-A immunoreactivity in the salivary gland. The discrepancy between the observations of the present study and those of Madsen et al2 may be attributed to the difference in sensitivity of immunohistochemical analysis between the 2 studies.

The prominent role of collectins in the lung seems to be that of a host defense mechanism against pathogens.1-3 The same role is likely to be present also in those organs that, similar to the lung, display direct contact between the mucosal surface and the external milieu, such as the eustachian tube,20 paranasal sinus,21 and vagina.22 The presence of SP-A at these mucosal surfaces, in close contact with numerous potentially harmful microorganisms, supports a role for SP-A in innate mucosal defense at these locations. Because the salivary gland ducts are exposed to the oral cavity, they are vulnerable to environmental insults from various microbial pathogens. The data presented in this study suggest up-regulation of SP-A mRNA in chronic sialadenitis, perhaps as a consequence of participation of SP-A in the innate mucosal defense in the salivary gland ducts. The localization of SP-A at an interface between the host and pathogens would be optimal sites to counter external pathogens and could help prevent the propagation of infections from the oral cavity to the salivary glands.

A number of researchers have found increased SP-A expression in the presence of inflammation. Sugahara et al23 found that intratracheal instillation of lipopolysaccharide in adult rats induces markedly increased expression of SP-A mRNA and SP-A protein production, while Cheng et al24 reported elevated SP-A and surfactant protein D levels in the airway epithelium of patients with mild, stable asthma. These findings are consistent with the results from the present study, which showed increased SP-A expression in the epithelium of salivary glands with chronic sialadenitis. These results suggest that elevated SP-A levels may be the consequence of inflammation.

However, decreased expression of SP-A has also been observed in individuals with disease and infection. Reduced levels of SP-A are observed in patients with severe bacterial infection and viral pneumonia,25 and functional depletion of SP-A is recognized in those with adult respiratory distress syndrome.26 These findings argue for the notion that impaired SP-A levels may be the cause of susceptibility to infection. Whether the change in SP-A levels is a cause or consequence of tissue inflammation should be a subject of further study.

In conclusion, in the salivary gland, there is differential expression of SP-A between inflammatory conditions and the healthy state. Although it is not clear whether the up-regulation of SP-A is a cause or a consequence of sialadenitis, we propose that altered SP-A levels in the inflamed salivary gland contribute to the host’s defense mechanism.

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References