Expression of Cathelicidin in Human Salivary Glands

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Background: Salivary secretions play a critical role in maintaining oral health via innate host defense mechanisms and secretion of secretory IgA. One of the antimicrobial peptides, LL-37, is the only cathelicidin protein that has yet been identified in humans. Cathelicidins are a family of peptides thought to provide an innate defensive barrier against a variety of potential microbial pathogens.

Objectives: To examine the expression of cathelicidin in human salivary glands and to investigate up-regulation of cathelicidin in inflammatory conditions.

Design: Reverse transcriptase–polymerase chain reaction and immunohistochemical staining were performed on 20 salivary gland tissues, 10 from normal salivary glands and 10 from glands with chronic sialadenitis.

Results: Cathelicidin messenger RNA transcripts were detected in the tissues from the normal salivary glands and the glands with chronic sialadenitis. The level of cathelicidin messenger RNA in glands with chronic sialadenitis was significantly increased compared with that in normal salivary glands. Cathelicidin protein was expressed in the glandular epithelium of the normal salivary glands and the glands with chronic sialadenitis.

Conclusion: The results indicate that cathelicidin might play an important role in innate host defense of human salivary glands.

EXTRACTION OF RNA

Tissue was homogenized in 1 mL of Trizol reagent (Gibco BRL, Tucson, Ariz), and RNA was extracted according to the manufacturer's instructions. Samples were air-dried and resuspended in water treated with diethyl pyrocarbonate and were kept on ice for immediate use or stored at −70°C for subsequent RNA studies. For RT-PCR–positive control of cathelicidin, lung epithelium was prepared in the same manner. Another portion was fixed with 4% paraformaldehyde in 0.1M phosphate-buffered saline (pH 7.4), stored overnight at 4°C, and then embedded in paraffin for immunohistochemical staining. Informed consent had been given by all patients. The tissue procurement procedures were approved by the institutional review board at Korea University.

IMMUNOHISTOCHEMICAL STAINING FOR CATHELICIDIN PROTEIN

The paraffin blocks were sliced into 5-µm-thick sections. Deparaffinization with xylene and rehydration with 100% and then 75% alcohol were done serially. Paraffin sections from the paraformaldehyde-fixed salivary tissues were treated with 3% hydrogen peroxide methanol to block endogenous peroxidase and were incubated with a rabbit polyclonal antibody to cathelicidin (a generous gift of Dr Ole Sorenson, Granulocyte Research Laboratory, National University Hospital, Copenhagen Denmark) was used at a dilution of 1:100 and incubated overnight at 4°C in a humidified chamber. Immunoreactive cathelicidin was visualized with a Vectastain Elite ABC Kit (Vector Lab Inc, Burlingame, Calif). Controls included the substitution of primary or secondary antibody with phosphate-buffered saline.

STATISTICAL ANALYSIS

Data were expressed as the mean±SEM. Comparisons of quantitative data between 2 groups were analyzed with the Mann-Whitney test. Differences were considered significant for P values less than .05.

RESULTS

The RT-PCR studies showed that salivary tissue contained mRNA encoding for cathelicidin. The PCR products from the salivary tissue had the size (570 bp) that was expected from the selected primers. The same-sized products from the salivary tissue had the size (570 bp) that was expected from the selected primers. The same-sized product was expressed in the positive control (Figure 1). There was significant difference in the amount of cathelicidin mRNA expression between the tissues of the chronic sialadenitis and normal salivary glands. The ratio was 2.69 ± 0.28 for the chronic sialadenitis gland group
and 0.36 ± 0.14 for the normal salivary gland group. The difference between the 2 groups was statistically significant ($P = .009$) (Figure 2).

**IMMUNOHISTOCHEMICAL STAINING OF CATHELICIDIN**

Immunostaining showed that cathelicidin expression was localized to the ductal cells and to the inflammatory cells of chronic sialoadenitis (Figure 3A) and normal salivary glands (Figure 3B); acinar cells were uniformly negative for cathelicidin staining (Figure 3D). There was no specific localization with negative control, confirming the specificity of the cathelicidin antibody (Figure 3C).

**COMMENT**

Innate immunity is important for the integrity of the host against potentially invasive pathogenic microorganisms in the environment. In contrast to highly specific adaptive immunity, the innate immune system provides a rapid and nonspecific response and thereby contributes to the first line of defense. Antibiotic peptides with broad antimicrobial activity are part of the innate immune system (nonadaptive immune system) and serve a key protective role in the host defense. They are acting as effector molecules with the capacity to kill a broad spectrum of microorganisms. The peptides are strategically located at sites exposed to microorganisms such as the airway and gastrointestinal tract and in phagocytes. The first peptide antibiotics were discovered in the 1980s when cecropins were isolated from insects and defensins were isolated from rabbit macrophages. Numerous antimicrobial peptide antibiotics occur in nature, and over a dozen have been identified in humans, including several salivary histatins, lactoferricin, 6α-defensins, 2B-defensins, and the human cationic antimicrobial protein hCAP18.

The hCAP18 protein (human cationic antimicrobial protein of 18 kd) is a newly described protein of human neutrophilic granulocytes, which belongs to the cathelicidin family of antimicrobial proteins. Members of this protein family share a common N-terminal sequence followed by a highly diverse antimicrobial, cationic C-terminus. The family of antimicrobial peptides, whose members contain this conserved N-terminus, are provisionally called the cathelicidins. Four cathelicidins have been identified in bovine neutrophils and 9 in porcine neutrophils. However, only 1 cathelicidin, hCAP18, is found in human neutrophilic granulocytes.

The hCAP18 protein is synthesized in neutrophil progenitor, myelocytes, and metamyelocytes in the bone marrow and stored in the peroxidase-negative granules.
of mature neutrophils. It is synthesized as an 18-kd proprotein from which a 5-kd C-terminal fragment, LL-37, bearing all of the hitherto known biological activity, is cleaved. The plasma level of cathelicidin is 1.18 µg/mL, which is severalfold higher than that for other specific granule proteins of neutrophils. The cathelicidin is present in plasma as high-molecular-weight complexes. The cathelicidin is processed to the antimicrobial peptide LL-37 by extracellular cleavage with protease. The cysteine-free peptide LL-37 can adopt an amphipathic α-helical conformation, has lipopolysaccharide-binding properties, and manifests antibacterial effect against a wide range of bacterial species through C-terminal domain. The bacterial-killing properties of cathelicidin are synergistic with lactoferrin and lysozyme. The promotor region of the cathelicidin gene contains potential binding site for transcription factors, the acute-phase response factor, and the nuclear factor for interleukin 6. Interleukin 6 may play an important role in modulating cathelicidin gene expression.

In the present study, we demonstrated the antimicrobial peptide cathelicidin mRNA and protein in human salivary glands. Cathelicidin is primarily detected in the duct cells of the gland, so cathelicidin can be one of the protection materials in salivary gland itself. Because ducts of the salivary glands are open to the oral environment, they are open to environmental insults from bacteria, viruses, and fungi. There is opportunity for retrograde infection into the gland, which is essentially a cul de sac. Cathelicidin could be active at the point of entry for oral bacteria, thus acting as a first-line host defense for this vulnerable space as well as having a functional role as a secreted protein in saliva. By immunohistochemical staining, the cathelicidin protein was found to be localized predominantly in the duct cells of salivary gland. This indicates that cathelicidin, which was previously found in the lung, squamous epithelia of the mouth, tongue, esophagus, and vagina, and nasopharynx, is constitutively expressed in the salivary gland, a site of constant microorganism challenge. By RT-PCR, the cathelicidin mRNA was detected in all cases of chronic sialadenitis and had an increased expression compared with the cathelicidin mRNA was detected in all cases of chronic sialadenitis and had an increased expression compared with the cathelicidin mRNA was detected in all cases of chronic sialadenitis and had an increased expression compared with the cathelicidin present in human neutrophils and plasma. Localizing expression of cathelicidin protein in human salivary glands and nasopharynx is of particular interest in view of the role of cathelicidin in antimicrobial immunity of the host.

In the present study, we showed the localizing expression of the human cathelicidin in salivary glands and up-regulation in the inflammatory conditions. This finding suggested the physiologic importance of cathelicidin in the defense of retrograde infection. Other functions for this broadly expressed peptide and possible expression of cathelicidin in saliva need to be evaluated.

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REFERENCES