Annals of Anatomy xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Annals of Anatomy

journal homepage: www.elsevier.de/aanat

The distribution of human surfactant proteins within the oral cavity and their role during infectious diseases of the gingiva

Martin Schicht [a,*,1], Christina Stengl [a,1], Sadettin Sel [b], Friedhelm Heinemann [c], Werner Götz [d], Anselm Petschelt [e], Matthias Pelka [e], Michael Scholz [a], Felix Rausch [a], Friedrich Paulsen [a], Lars Bräuer [a]

[a] Department of Anatomy II, Friedrich-Alexander-University Erlangen-Nürnberg, Universitätsstr. 19, Erlangen 91054, Germany
[b] Clinic of Ophthalmology, Ruprecht-Karls-University Heidelberg, Im Neuenheimer Feld 400, Heidelberg 69120, Germany
[c] Department of Prosthodontics, Gerodontology and Dental Materials, Dental School, University medicine of Greifswald, Rotgerberstr 8, Greifswald 17475, Germany
[d] Labor für Oralbiologische Grundlagenforschung, Poliklinik für Kieferorthopädie der Rheinischen Friedrich-Wilhelms-Universität Bonn, Welschnonnenstrasse 17, Bonn 53111, Germany
[e] Dental Clinic 1, University Hospital of Erlangen, Glücksstraße 11, Erlangen 91054, Germany

ARTICLE INFO

Article history:
Received 2 April 2014
Received in revised form 13 May 2014
Accepted 29 May 2014
Available online xxx

Keywords:
Surfactant proteins
Gingivitis
Periodontitis
Saliva

ABSTRACT

The oral cavity with the teeth and the surrounding gingival epithelium, the periodontium, the salivary glands and other structures are open to the oral environment and thus exposed to multiple microbiological and pathogenic influences. To prevent permanent inflammatory processes such as gingivitis or periodontitis an efficient defense system is essential to ensure healthy and physiological function of the oral cavity and other interacting organic systems. Surfactant proteins (SPs), originally found in pulmonary tissue are important factors of the immune system and beyond this, support the stability and rheology of gas or fluid interfaces. This study aimed to analyze the distribution of surfactant proteins by means of Western blot and immunohistochemistry in salivary glands as well as in healthy and pathological saliva. The different expression patterns of SP-A, -B, -C and -D in healthy and pathological (periodontitis) saliva were determined using ELISA quantification. One further objective of the study was the first detection of two recent discovered proteins belonging to the surfactant protein family within human salivary glands and saliva. The results of the study reveal differences in protein expression of SP-A, -B, -C and -D within healthy and pathologic saliva. The concentration of the surfactant proteins SP-A, SP-C and SP-D is increased in saliva of people suffering from periodontal diseases, whereas by contrast, SP-B shows an opposite expression pattern. Furthermore, the results evidence the presence of SP-G and SP-H within saliva and salivary glands for the first time.

1. Introduction

Gingival and periodontal diseases are inflammatory reactions of the tissues surrounding the teeth and can affect up to 90% of the worldwide population (Pihlstrom et al., 2005). They are causes by microorganisms that colonize the adherent dental plaque adjacent to the gingiva. Besides the pathogenic microorganisms, environmental or genetic factors, especially smoking, are risk factors for this disease (Pihlstrom et al., 2005). There are different levels of periodontal diseases. Gingivitis is the initial form of inflammation and does not destroy the underlying supporting structures of the teeth. It causes swollen, red and easily bleeding gum but is fully reversible (Pihlstrom et al., 2005). These gingival reactions are visible answers of the local immune system. Gingivitis can progress to periodontitis, which is an inflammation affecting the periodontal ligaments and the alveolar bone. Because of the destruction of the gingival fibers, the gum tissues separate from teeth and create a periodontal pocket. Microorganisms which prefer anaerobic environment such as *Porphyromonas gingivalis* or *Treponema denticola* (Holt and Ebersole, 2005) find perfect conditions to proliferate and cause the progression of the inflammation. This leads to loss of desmodontal fibers, alveolar bone and loosening of teeth. The final stage of this disease is the loss of teeth apart from those healthy teeth. In addition, periodontitis can be associated with systemic diseases, such as cardiovascular diseases (Desvarieux et al., 2005), rheumatoid

* Corresponding author. Tel.: +49 9131 8526733.
[1] Contributed equally to this work.

http://dx.doi.org/10.1016/j.aanat.2014.05.040
0940-9602/

arthritis (de Pablo et al., 2009) and harmful pregnancy outcomes (Xiong et al., 2006). An effective defense system combining innate immunity and salivary flow is necessary to avoid or heal the infection (Lee et al., 2005).

Saliva is the secretion of exocrine glands (parotid, submandibular, sublingual, lingual, labial, palatinal, buccal and molar), among which the parotid and submandibular glands are responsible for the largest share of saliva. It consists of roughly 99% water, and a huge variety of electrolytes, proteins, immunoglobulins, antimicrobial peptides and mucosal glycoproteins (Edgar, 1992; Humphrey and Williamson, 2001; Berkovitz et al., 2002; Ferraris, 2006). The main function of saliva, besides oral lubrication and oral predigestion, is building an immunological barrier against the invasion of microorganisms into the gingival epithelium. A variety of antimicrobial substances and surface active proteins have been described to be components of saliva, amongst them surfactant proteins, originally known from the lung (Bräuer et al., 2009).

The surfactant proteins SP-A, SP-B, SP-C and SP-D have been described in detail in research related to the lung. Surface activity and immunological functions in the non-specific and specific immune defense systems have been ascribed to them. SP-A and SP-D are representatives of the C-type lectin-family, where numerous other molecules of known immunological function can be counted. In accordance with the current understanding of the mechanism of C-type collections, exposed microbiological carbohydrates (on the surface of diverse microorganisms) bind to a carbohydrate recognition domain inherent to the proteins. This is the way opsonisation and acceleration of the defense reaction to the microorganism is achieved (van de Wetering et al., 2004; Wright, 2005; Kishore et al., 2006).

In the meantime the presence of SP-A and SP-D with regard to their immunological function has been detected in different extrapulmonal tissues, such as human nasal epithelium, the digestive tract, mesentery, the lacrimal system, the major salivary glands and the gingival epithelium (Bourbon and Chailley-Heu, 2001; Kim et al., 2007; Bräuer et al., 2007a, 2009; Schicht et al., 2013).

In contrast to SP-A and SP-D, the small hydrophobic surfactant proteins SP-B and SP-C seem to be essential components during formation of surface active monolayers and the stabilization of an air–liquid interface (Curstedt et al., 1987; Notter et al., 1987; Yu and Possmayer, 1990). SP-B and SP-C have also been observed in several tissues and fluids outside the lung such as in tears, the lacrimal apparatus, the major salivary glands and saliva (Bräuer et al., 2007b, 2009). Reduction of surface tension, rheological functions and assistance during pellicle formation facilitated by the surfactant proteins would also have great implications for the physiological function of the oral epithelium and the gingiva.

While working on the characterization of the salivary surfactant proteins we recognized different expression patterns in samples of patients suffering from periodontal diseases compared to healthy saliva. So the aim of this study was to further investigate possible differences in the quantity of the SPs in healthy saliva compared to saliva from patients with pathologically altered periodontal tissues. Recent studies revealed two novel proteins belonging to the family of surfactant proteins namely SP-G and SP-H that have been detected in different tissues amongst them in human lung (Rausch et al., 2012; Schicht et al., 2014). In this context one further objective of the study was to investigate salivary glands and saliva for the possible presence of SP-G and SP-H.

2. Materials and methods

The study was conducted in compliance with Institutional Review Board regulations, informed consent regulations according to the provisions of the Declaration of Helsinki. The institutional ethics committee confirmed the necessary approval for the collection and investigation of saliva samples (9_12B).

2.1. Saliva and tissue

Saliva was collected from 80 different patients (51 females and 29 males, aged 23–76 years). Forty were healthy (*h*) volunteers, 40 suffered from periodontal disease (*pd*). All patients and volunteers underwent periodontal screening (PS) as described by (Ziebolz et al., 2011). Volunteers with PS index of 0 were considered to be healthy, patients with PS index 3 and 4 were considered to be pathological and therefore subdivided into the respective groups (*he* and *pd*). A capillary tube was directly placed at the orifice of the submandibular ducts. Collected saliva samples were immediately frozen and stored at −80 °C for use in Western blot analysis and ELISA. Submandibular glands as well as human lung (positive control) was obtained from cadavers donated to the Department of Anatomy and fixed as previously described by Tektas et al (Tektas et al., 2012).

2.2. Antibodies

The following antibodies were used in Western blot analysis: rabbit anti human SP-G and SP-H: mouse anti human α-actin, Santa Cruz (1:1000); secondary antibodies were used as follows: Goat anti Mouse HRP, Dianova (1:5000); Goat anti Rabbit HRP, Dako (1:5000).

2.3. Western blot analysis

For Western blot analysis, different samples of saliva (*he* as well as *pd*) were investigated. The procedure was performed as previously described by Posa et al. considering the following modification (Posa et al., 2012). The protein content of these collected saliva samples, was measured with a protein assay based on the Bradford dye-binding procedure (BioRad, Hercules, CA). Total protein (20 μg) was then analyzed by Western blot. Proteins were resolved by reducing 15% SDS-polyacrylamide gel electrophoresis, electrophoretically transferred at room temperature for 1 h at 0.8 mA/cm^2 onto 0.1 μm pore size nitrocellulose membranes and fixed with 0.2% glutaraldehyde in phosphate-buffered saline for 30 min. Bands were detected with primary antibodies to SP-G (1:250), SP-H (1:250) and secondary antibodies (anti-rabbit/anti-mouse IgG, respectively, conjugated to horseradish peroxidase, 1:5000) applying chemiluminescence (ECL-Plus; Amersham-Pharmacia, Uppsala, Sweden). Human lung was used as the control. The molecular weights of the detected protein bands were estimated using standard proteins (Prestained Protein Ladder, Fermentas, St. Leon-Rot, Germany) ranging from 11 to 170 kDa.

2.4. IHC

For immunohistochemistry, tissue specimens from healthy tissues of cadavers were embedded in paraffin, sectioned (6 μm) and dewaxed. Immunohistochemical staining was performed with the polyclonal antibody against SP-G and SP-H (Rausch et al., 2012; Schicht et al., 2014). Antigen retrieval was performed by microwave pretreatment for 10 min and non-specific binding was inhibited by incubation with porcine normal serum (Dako) 1:5 in Tris-buffered saline (TBS). Each primary antibody (1:200) was applied overnight at room temperature. The secondary antibodies (1:300) were incubated at room temperature for at least 4 h. Visualization was achieved with aminoethylcarbazole (AEC) for at least 5 min. Red stained areas within the tissues indicate a positive antibody reaction. After counterstaining with hemalum, the sections were mounted in Aquatex (Boehringer, Mannheim,

Please cite this article in press as: Schicht, M., et al., The distribution of human surfactant proteins within the oral cavity and their role during infectious diseases of the gingiva. Ann. Anatomy (2014), http://dx.doi.org/10.1016/j.aanat.2014.05.040