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SURFACTANT AND ITS ROLE IN THE UPPER RESPIRATORY SYSTEM AND EUSTACHIAN TUBE

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Abstract

Surfactant research was originally directed toward lung mechanics, however, with growing information on the biology of the surfactant system it has expanded beyond the borders of basic physiology. The research has become interdisciplinary, not only considering aspects of lungs biology relevant for breathing, but also those aspects relevant for airway defence.

Surfactant consists mainly of phospholipids that lower the alveolar surface tension to prevent lung collapse at expiration. They also support mechanical elimination of inhaled pathogens by reducing the viscosity of airway mucus. Approximately 8-10% of surfactant is made up of proteins. Among them, specific proteins SP-A and SP-D play a crucial role in the innate defence system. They belong to collectins family and serve as the first step in immune response to inhaled pathogens. In limited extent, SP-B and SP-C are also involved in immunomodulation.

Although numerous studies have focused on the physiological function of surfactant in the lower airways, relatively little is known about its role in the upper respiratory system. Identification of lamellar bodies in ciliated epithelium of the upper airways indicates that surfactant may have a role in normal sinonasal function and pathology. Decreased levels of the main component of surfactant, phospholipids, have been implicated in atrophic rhinitis and altered levels of surfactant proteins have been observed in a number of respiratory tract diseases. The pattern of inflammation in the upper respiratory tract generally appears to parallel that in the lower airways and nowadays upper respiratory disease and lower airway disease are considered as two manifestations of one pathological process. Therefore, surfactant proteins may play a significant role in the upper respiratory tract diseases.

In addition, surfactant has been identified in the Eustachian tube where it helps to lower the opening pressure between nasopharynx and middle ear. The alterations in surfactant levels may adversely affect Eustachian tube function and contribute to chronic ear infection.

The review summarizes the current knowledge on the presence and the role of surfactant in the upper respiratory system and Eustachian tube.

Key words: surfactant, specific proteins, upper airways, otitis media, Eustachian tube

INTRODUCTION

Pulmonary surfactant, a complex of lipids and proteins lining the alveolar surface, has two crucial roles in respiratory function. It reduces surface tension at the air liquid interphase, facilitating gas exchange and alveolar stability during breathing and interacts with the airway defence system (1).

In addition to the lung, surfactant-like material has been identified in many other human tissues. Lamellar bodies or phospholipids and surfactant proteins have been detected in non-pulmonary sites including upper respiratory tract, Eustachian tube, middle ear, gastrointestinal tract, salivary glands, brain, trachea, lacrimal glands, heart, kidney, pancreas, and male and female urogenital tract (2-7).

Composition of surfactant

Surfactant is composed of 85-90% lipids, about 10% proteins and 2% carbohydrates. The principal lipid constituents of surfactant are phospholipids. Phosphatidylcholine

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(PC) species comprise about 75% of surfactant phospholipids. Nearly half of the PC content is dipalmitoylphosphatidylcholine (DPPC), which is the major component of surfactant and also principal surface tension reducing compound. Up to half of the intra-alveolar content of DPPC is present in the monolayer at the air-liquid interphase. Other phospholipids include phosphatidylglycerol (~12%), phosphatidylethanolamine (~5%), phosphatidylinositol (~4%), phosphatidylserine (~1.5%), sphingomyelin (~1%), and lysophospholipid (<1%). Cholesterol is major neutral lipid, and constitutes about 6-8% of the total lipids (8).

Optimal surfactant function requires the presence of four specific proteins known as SP-A, SP-B, SP-C and SP-D. Hydrophobic surfactant proteins SP-B and SP-C facilitate the adsorption of phospholipids at the air-liquid interphase, where they reduce surface tension. Hydrophillic SP-A and SP-D play a role in the pulmonary host-defence system (9).

Metabolism of surfactant

Both surfactant lipids and proteins are produced in the alveolar type II cells. The phospholipids and three of four surfactant specific proteins, except SP-C, are also synthesized in the airways in non-ciliated epithelial Clara cells. Surfactant components were also found in secretory cells of airway submucosal glands (10).

Inside the cells, the surfactant components are stored in dense, multilayered membrane structures – the lamellar bodies. Lamellar bodies are excreted into the alveoli and converted into a lattice-like structure of tubular lipid double-layers, called tubular myelin, from which the monolayer at the air-liquid interphase is formed. Formation of these structures and their transformation is facilitated by surfactant proteins (10).

A major clearance pathway for surfactant is an uptake and reutilization by the type II cells. A significant fraction of surfactant is degraded by alveolar macrophages, with minor amounts moving up to the airways and across the epithelial-endothelial barrier into the blood stream (11).

The immunological role of surfactant

The main function of the ***phospholipids***, primarily DPPC, is to lower the surface tension at the air-liquid interphase, however, they also possess immunomodulating properties. Surfactant lipids have been shown to suppress the release of inflammatory cytokines and prostanoids by monocytes and to inhibit activation of both T and B lymphocytes. They are able to quench surface free radical activity of mineral dust particles and reduce their toxicity in vitro (12). Moreover, the surfactant phospholipids coat the gel layer to reduce the surface tension, decrease mucus viscosity and enhance the elimination of inhaled pathogens (13).

The hydrophobic proteins ***SP-B and SP-C*** have been characterized extensively for their ability to affect lamellar body formation, secretion and creation of the surfactant monolayer that is critical to the lowering surface tension at the air-liquid interphase. Their role is crucial as children born with inherited SP-B and/or SP-C deficiency usually develop acute respiratory failure non-responding to exogenous surfactant replacement (14).

Increasing evidence indicates that SP-B and SP-C are also involved in immunomodulation that is critical for the host defence of the airways. For example, SP-B reduces inflammatory response in the lungs to bacterial lipopolysaccharide (15). In transgenic mice reduction in SP-B expression and the associated abnormalities in reducing surface tension evoked an inflammatory response in alveolar macrophages and alveolar type II cells (16). SP-C-deficient mice are susceptible to bacterial and viral infections and they suffer from excessive inflammation (17). Moreover, surfactant in combination with hydrophobic SP-B and SP-C possesses viscoelastic and rheologic properties that enhance mucociliary clearance (18). It accelerates ciliary beat frequency, decreases mucus viscosity and improves particle clearance from the lungs (19).

SP-A and SP-D are members of collectins family that belong to C-type lectins named for their aminoterminal collagen-like region and carboxyterminal or carbohydrate recognition domain (CRD). The collectins participate in innate immunity in the period before induction of an antibody-mediated response. These proteins selectively recognize and bind via their CRD to carbohydrate, but also lipopolysaccharide and protein moieties on the surface of bacteria, fungi, viruses and allergens. This binding is followed by interactions with dendritic cells or macrophages and leads to opsonization and pathogen clearance, complement activation, and modulation of leukocyte function including chemotaxis and subsequent cytokine and/or chemokine responses (20, 21).

Taken together, SP-A and SP-D are critical in the initial interaction, recognition, processing and subsequent adaptive immune response for a wide number of inhaled pathogens. In addition, SP-A and SP-D promote apoptotic cell uptake by innate immune cells and regulate cytokine and free radical production (9).

Several studies have shown that SP-A and SP-D bind to and inactivate wide range of pathogens, such as *Staphylococcus aureus*, *Haemophilus influenzae* type A, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and a group A streptococci, *Klebsiella pneumoniae*, *Aspergillus*, *Salmonella minnesota*, *E. coli*, herpers simplex virus, infuenza virus, *M. tuberculosis*, *Pneumocystis carinii*, *Mycoplasma pneumoniae* (22, 23).

Beside binding to pathogens and affecting antigen processing, SP-A and SP-D affect immunoglobulin E binding to the allergen and cause a shift in the polarization of T-lymphocytes subpopulations Th1 and Th2 (13). In addition, topical application of SP-A and SP-D have been shown to decrease immunoglobulin E levels and reduce eosinophilia in mouse model of allergic bronchopulmonary aspergillosis and to cause a marked shift from a pathogenic Th2 profile to a protective Th1 cytokine pattern (22).

While SP-D not being associated with surfactant lipids is solely included in immune processes, surfactant protein A cooperates with SP-B in formation of the tubular myelin and the surface film (24). It does not have an essential role in reduction of surface tension, since mice deficient in SP-A will survive and have normal compliance and lung volumes (25). Moreover, SP-A increases resistance of surfactant to some inhibitors (26).

SURFACTANT IN THE UPPER RESPIRATORY SYSTEM

The nose and sinuses play an important role in the first line-defence of the respiratory tract. By warming up, humidifying and filtering incoming air the nose and sinuses are essential in the protection and homeostasis of lower airways (27). As in lower airways, surfactant in upper respiratory system may also be impaired by inhaled noxious agents (28).

Despite their differences, both the upper and the lower airways are crucial in the body's defences against inhaled pathogens, and the pattern of inflammation in the upper respiratory tract generally appear to parallel that in the lower airways (29). Recently, the concept of "united airway disease " or "one linked airway disease" has been proposed (30). In this concept, upper airway disease and lower airway disease are considered as two manifestations of one pathological process.

The close relationship between asthma, allergic and nonallergic rhinosinusitis and nasal polyps has been acknowledged for many years. It has been estimated that approximately 90% of allergic asthmatics suffer from rhinitis, and around 30% of rhinitis patients suffer from asthma (31). Individuals with asthma sensitive to the ingestion of aspirin may suffer from nasal polyps as part of the disease process (32). Allergic fungal rhinosinusitis is considered to be the upper airway correlate to allergic bronchopulmonary aspergillosis (33). Patients with cystic fibrosis invariably develop chron-

ic rhinosinusitis (CRS) in addition to their pulmonary disease. This is through the similar mechanism of inspissated mucus, impaired mucociliary clearance, and persistent bacterial infections and inflammation (34). Several studies report the prevalence of chronic nasal symptoms in patients with chronic obstructive pulmonary disease as 40-70% (35). On the other hand, there is 40% prevalence of lower airway disease in patients with CRS (36) and, interestingly, 70% of those patients were first diagnosed as having lower airway disease. Thus, the lungs and the paranasal sinuses share contact with inhaled pathogens and include many of the same morphological and functional properties.

Surfactant phospholipids in the upper airways

Although numerous studies have focused on the nature and defensive role of surfactant in the lower airways, relatively little is known about its role in the upper respiratory system.

Identification of lamellar bodies in ciliated pseudostratified epithelium of the upper airways (5, 6) indicates that surfactant may have a role in normal sinonasal function and pathology. These lipid storage and secretory organelles possibly undergo exocytosis and organize to form surfactant in the lumen of the sinonasal cavity in a fashion similar to that in the lower airways. Biochemical analysis of the nasal aspirate in healthy individuals revealed the presence of phospholipids constituting surfactant as phosphatidylcholine, phosphatidylethanolamine, sphingomyelin and other phospholipids (37). It was observed that phosphatidylcholine constituted ~75% phospholipids of the nasal aspirate, while phosphatidyethanolamine constituted ~15 %, sphingomyelin ~5% and other phospholipids 4% (Fig.1).

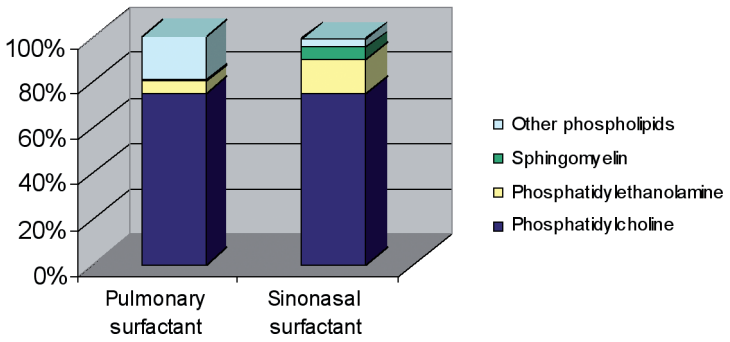

Fig 1. Differences in phospholipid content of pulmonary and sinonasal surfactants

This phospholipids profile corresponds with that observed in the lung wash (38) and in the Eustachian tube and nose (39) in healthy humans.

In patients with primary atrophic rhinitis compared to the healthy people total phospholipids content decreases and its profile changes (37). The changes are characterized by significant decrease in phosphatidylcholine and increase in phosphatidylethanolamine and sphingomyelin. This is in agreement with the study done by Gunther et al. (40) who demonstrated reduced phospholipids concentrations in the bronchoalveolar lavage fluid in all patients with inflammatory lung injury. The compositional changes in the phospholipids profile are similar to that observed in premature infants with neonatal respiratory distress syndrome and in acute pulmonary inflammation in adult respiratory distress syndrome and/or pneumonia (40, 41).

Surfactant proteins in the upper airways

Recent studies identified surfactant proteins and their messenger RNA (mRNA) in normal and diseased sinonasal tissue (42, 43). Immunolocalization of surfactant specific proteins demonstrates their presence in pseudostratified ciliated epithelium and submucosal secretory ducts of sinonasal mucosa (44, 45).

The location of SP-A and D is consistent with the role of these proteins in the innate defence against pathogens at sites of potential invasion of microorganisms. The discovery of surfactant production and secretion by sinonasal mucosa indicates that initial contact and interaction between pathogens and surfactant proteins occurs relatively early after inhalation and deposition into the mucus of the upper respiratory tract.

In sinus mucosal biopsies from patients with cystic fibrosis hydrophillic SP-A and SP-D (42), as well SP-B mRNA (46), were up-regulated when compared with healthy controls. The upregulation is likely due to the substantial bacterial infections that accompany this form of chronic rhinosinusitis (CRS), although undetermined genetic factors and immunologic dysfunction could also play a role. *Pseudomonas aerigunosa* invariably colonizes and infects the sinuses of patients with CF and has been shown to degrade surfactant components including surfactant proteins (47). This may result in a compensatory response at the cellular level to increase expression of SP mRNA and surfactant production. However, content of SP-A and D in bronchoalveolar lavage fluid was reduced in CF patients and it was even lower during an active infection (48). In these studies, protein levels only and not the cellular mRNA were measured. It is possible, that in CF patients there is an upregulation of SP-A and D gene expression and subsequent protein production, but these are rapidly degraded in the presence of bacteria.

Lee et al. (49) demonstrated upregulation of SP-A mRNA and more intense expression of SP-A protein in paranasal sinus mucosa of patients with chronic rhinosinusitis than in healthy control. It indicates that the SP-A gene in paranasal sinus mucosa is not only constitutively expressed, but it is also upregulated during inflammation. The upregulation of SP-A mRNA and SP-A protein in the paranasal sinus mucosa in patients with CRS suggests its role in the local defence mechanism of the paranasal sinus mucosa. These findings are similar to that in patients with chronic allergic rhinosinusitis (50). Moreover, the degree of SP-A mRNA expression correlated with severity of disease measured by Rhinitis Symptom Utility Index in patients with allergic rhinitis symptoms. Linking SP-A expression to the severity of nasal symptoms, sneezing and running nose suggests that SP-A may be an important molecule in local nasal inflammation as well, but additional work is necessary to determine whether SP-A elevation is a reaction to local allergy or a mediator of it. In addition, the expression of SP-A and SP-D is positively influenced by the degree of cell differentiation into mucociliary epithelium (45).

EFFECT OF SURFACTANT ON EUSTACHIAN TUBE

Eustachian tube (ET) connects the middle ear with the nasal cavity and it is important for ventilation, protection and clearance of the middle ear (ME). The structure of the ET is similar to other respiratory airways in which the lumen of the tube is bound by a thin fluid layer at the mucosal surface and is surrounded by cartilaginous and muscular elements. Under physiological conditions ET exists in a “collapsed” configuration that protects the middle ear from nasopharyngeal secretions. However, ET is also responsible for maintaining ambient middle ear pressures and clearing the ME fluid into the nasopharynx. The pressure-regulating and clearance functions require an

open ET in which the resistance to air and fluid flow is minimal. Opening pressure is assumed to reflect the pressure that is needed to overcome all closing forces, including the solid-to-solid adhesion of the ET walls (luminal forces) and pressure of the cartilage and other surrounding tissues (extraluminal forces). The closing pressure is believed to reflect the extraluminal forces, so it has passive ventilatory function (51). Thus, ET dysfunction and the resulting disease complications can develop when the tube is excessively patent or cannot be readily open.

Reduced tubal patency is regarded as possible factor in the development of middle ear disease. Otitis media (OM) is a common childhood disease that includes inflammation of the middle ear mucosa and an accumulation of fluid within the ME. By age of 3 years, a significant number of children (33%) experience more than three episodes of OM (52). The persistence of OM often results in hearing loss, with possible effects on language acquisition, speech production, and social and educational development (53). Although bacterial or viral infections and nasal allergies contribute to the onset of OM, the development of persistent OM is associated with a functional impairment of the Eustachian tube (51).

The role of surfactant in ET function has been questioned for several years. As early as in 1963, Flisberg et al. (54) suggested that the surface activity of the mucous lining of the tubal lumen may be important in tubal opening (Fig.2).

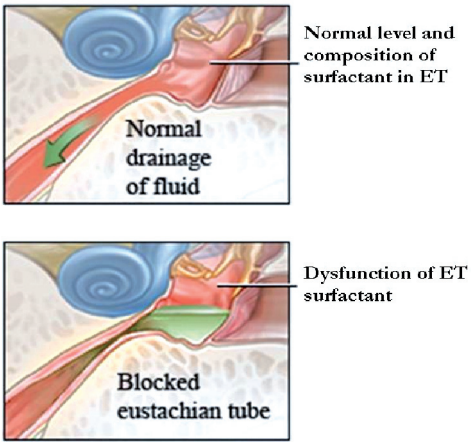

Fig. 2 The role of surface-active material in Eustachian tube (modified according to http://ptolentinobioresearch.wikispaces.com). ET – Eustachian tube

Since then, surface tension-lowering factors (surfactants) have been identified in both the middle ear and ET, both in animals (55) and humans (56). Surfactant-producing cells morphologically similar to the alveolar type II cells in the lungs were found in the dorsal part of the ET (57).

ET surfactant is composed of specific surfactant proteins (58) and a mixture of predominantly phospholipids, especially phosphatidylcholines and sphingomyelins (59). Phospholipids are known to reduce the surface tension at an air-aqueous interphase. However, because the ET is normally closed, the ET surfactant covering the epithelium is supposed to act as a release agent by preventing solid-to-solid adhesion (59). Grace et al. (56) compared the phospholipids content of middle ear effusions resulting from ET obstruction in adult patients with that of children with secretoric otitis media. In both groups, surface tension-lowering substances were isolated but the composition

was different from adults having a higher amount of sphingomyelin. In other study, a higher sphingomyelin/phosphatidylcholine ratio was present in children with secretoric otitis media than in those without OM (60) indicating a lower degree of surface-lowering properties.

Several recent studies have investigated the effect of exogenous surfactant on ET mechanics. Exogenous surfactant reduces opening pressure of the ET (61), also under pathological conditions in patients with otitis media (62, 63). It also seems to improve clearance function of the ET as a significant enhancement of mucociliary transport has been observed after the application of surfactant, both in vitro and in vivo (64). Theoretically, enrichment of administered surfactant by substances increasing its resistance (65) or having antimicrobial effect (66) could improve efficacy of such therapy.

Thus, the presence of a sufficient quantity and quality of ET surfactant may be an important determinant of ET functions and mechanics. Dysfunction of ET surfactant could be a possible factor in the development of serous otitis media.

CONCLUSION

Although numerous studies have focused on the nature and defensive role of surfactant in the lower airways, relatively little is known about its role in the upper respiratory system.

The lamellar body arrangement of phospholipids has now been demonstrated in the both normal and diseased sinus tissue, resulting in the implication that these structures may play a crucial role in the regulation of mucus viscosity and in mucociliary clearance against inhaled pathogens as well. Decreased levels of phospholipids have been found in atrophic rhinitis. Surfactant proteins make up a relatively small portion of surfactant, but appear to have an important role especially in innate immunity. They are crucial in the initial interaction, recognition, processing, and subsequent adaptive immune responses for a wide variety of inhaled pathogens and allergens. Presence of surfactant proteins in a variety of normal and diseased sinonasal tissue indicates that these proteins may play a significant role in physiology and pathophysiology of sinonasal diseases. Understanding the role of surfactant proteins in diseased and healthy states may help to develop novel treatments for sinonasal pathologies.

In addition, surfactant has been identified in the Eustachian tube where it helps to lower the opening pressure between the nasopharynx and middle ear. Therefore, alteration in surfactant levels may adversely affect Eustachian tube function and contribute to chronic ear infection.

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