Respiratory Physiology & Neurobiology 209 (2015) 95–105

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Pulmonary surfactant in the airway physiology: A direct relaxing effect on the smooth muscle☆

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ARTICLE INFO

Article history:
Accepted 5 January 2015
Available online 10 January 2015

Keywords:
Pulmonary surfactant
Upper and lower airways
Smooth muscle
Relaxation
Obstruction

ABSTRACT

Beside alveoli, surface active material plays an important role in the airway physiology. In the upper airways it primarily serves in local defense. Lower airway surfactant stabilizes peripheral airways, provides the transport and defense, has barrier and anti-edematous functions, and possesses direct relaxant effect on the smooth muscle. We tested *in vitro* the effect of two surfactant preparations Curosurf® and Alveofact® on the precontracted smooth muscle of intra- and extra-pulmonary airways. Relaxation was more pronounced for lung tissue strip containing bronchial smooth muscle as the primary site of surfactant effect. The study does not confirm the participation of ATP-dependent potassium channels and cAMP-regulated epithelial chloride channels known as CFTR chloride channels, or nitric oxide involvement in contractile response of smooth muscle to surfactant.By controlling wall thickness and airway diameter, pulmonary surfactant is an important component of airway physiology. Thus, surfactant dysfunction may be included in pathophysiology of asthma, COPD, or other diseases with bronchial obstruction.

1. Introduction

Pulmonary surfactant is a complex mixture of phospholipids and proteins that lines the inner surface of the respiratory system. Its major role is to reduce surface tension in the alveoli and prevent lung collapse at the end-expiration (Speer et al., 2013). Moreover, surfactant helps to keep the small airways open and regulates pulmonary host defense and immune responses (Chroneos et al., 2010).

Surfactant is composed of 85–90% lipids, about 10% proteins and 2% carbohydrates. The composition is very complex with at least fifty different phospholipids (Curstedt et al., 2013). The principal lipid constituents of surfactant are phospholipids. Phosphatidylcholine (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. Besides DPPC, surfactant contains a variety of minor lipids, such as plasmalogens, polyunsaturated fatty acid-containing phospholipids, and cholesterol (Rustow et al., 1994; Rudiger et al., 2005).

Optimal surfactant function requires the presence of four specific proteins known as SP-A, SP-B, SP-C and SP-D. Hydrophilic SP-A

☆ This paper is part of a special issue entitled "Molecular basis of ventilatory disorders" guest-edited by Dr. Mietek Pokorski.

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http://dx.doi.org/10.1016/j.resp.2015.01.004
1569-9048/

and SP-D play a role in the pulmonary host-defense system (Pastva et al., 2007). Hydrophobic surfactant proteins SP-B and SP-C facilitate the adsorption of phospholipids at the air–liquid interphase and they are crucial for alveolar stability at the end-expiration (Almlén et al., 2008).

Both surfactant lipids and proteins are produced by the alveolar type II cells. Inside the cells, surfactant components are stored in dense multi-layered membrane structures – the lamellar bodies (Walski et al., 2009). Lamellar bodies are excreted into the alveoli and converted in lattice-like structures of tubular lipid double-layers, called tubular myelin. Tubular myelin forms the monolayer at the air/liquid interphase. Formation of these structures and their transformation is facilitated by surfactant proteins (Andreeva et al., 2007).

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 (Wright and Dobbs, 1991).

In addition to the lungs, surfactant-like material has been identified in many other human tissues (Dutton et al., 1999; Madsen et al., 2000; Lin et al., 2001; Madsen et al., 2003) including the upper airways (Svane-Knudsen et al., 1990; Woodworth et al., 2005).

2. Surfactant in the upper airways

The nose and sinuses play an important role in the first line defense of the respiratory system. By warming up, humidifying and filtering incoming air, the nose and sinuses are essential in the protection and homeostasis of the lower airways (Hens and Hellings, 2006). Both upper and lower airways are crucial in the body's defense against inhaled pathogens, and despite the differences, the pattern of inflammation in the upper respiratory system generally appear to parallel that in the lower airways. Recently, the concept of "united airway disease" or "one linked airway disease" has been proposed (Passalacqua et al., 2001). 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 (Bachert et al., 2006). Individuals with asthma sensitive to the ingestion of aspirin may suffer from nasal polyps as a part of the disease process (Farooque and Lee, 2009). Allergic fungal rhinosinusitis is the upper airway correlate to allergic broncho-pulmonary aspergillosis (Pakdaman et al., 2011). Patients with cystic fibrosis invariably develop chronic 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 (Boari and de Castro Júnior, 2005). Several studies report the prevalence of chronic nasal symptoms in patients with chronic obstructive pulmonary disease as 40–70% (Roberts et al., 2003). On the other hand, there is 40% prevalence of lower airway disease in patients with CRS (Kim et al., 2007a, 2007b) and 70% of those patients were first diagnosed for 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.

2.1. 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 (Svane-Knudsen et al., 1990; Woodworth et al., 2005) 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 (Sayed et al., 2000). It was observed that phosphatidylcholine constituted ~75% phospholipids of the nasal aspirate, while phosphatidylethanolamine constituted ~15%, sphingomyelin ~5% and other phospholipids 4%. This phospholipid profile corresponds to that observed in the lung wash (King and Clements, 1972) and in the Eustachian tube in healthy humans (Hills, 1984; Calkovsky and Hajtman, 2007).

In patients with primary atrophic rhinitis compared to the healthy individuals total phospholipids content decreases and its profile changes (Sayed et al., 2000). The changes are characterized by the significant decrease in phosphatidylcholine and the increase in phosphatidylethanolamine and sphingomyelin. This is in agreement with the study of Günther et al. (1996) 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 those observed in premature infants with neonatal respiratory distress syndrome and in acute pulmonary inflammation in adult respiratory distress syndrome and/or pneumonia (Gregory et al., 1991; Günther et al., 1996).

2.2. Surfactant proteins in the upper airways

Recent studies identified surfactant proteins and their messenger RNA (mRNA) in normal and diseased sinonasal tissue (Woodworth et al., 2007a, 2007b; Schicht et al., 2013). Immunolocalization of surfactant specific proteins demonstrates their presence in pseudostratified ciliated epithelium and submucosal secretory ducts of sinonasal mucosa (Woodworth et al., 2006; Kim et al., 2007a, 2007b).

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

In sinus mucosal biopsies from patients with cystic fibrosis hydrophilic SP-A and SP-D (Woodworth et al., 2007a) as well SP-B mRNA (Woodworth et al., 2007c) were upregulated 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 aeruginosa* invariably colonizes and infects the sinuses of patients with CF and has been shown to degrade surfactant components including surfactant proteins (Beatty et al., 2005). 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 (Noah et al., 2003). In these studies, only protein levels, 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.