

EDITORIAL

The role of pulmonary surfactant in the asthmatic response

The importance of lung surfactant function has become widely appreciated in the context of neonatal Respiratory Distress Syndrome (RDS). Preterm infants born with developmentally immature lungs suffer from a primary deficiency of surfactant, which leads to alveolar instability, atelectasis and severe respiratory failure. One of the major success stories of respiratory care over the past decade has been treating RDS with exogenous surfactant. However, the dramatic improvement in survival of critically ill infants less than 30 weeks gestation has in many respects overshadowed the importance of adequate surfactant function to optimal lung function throughout life. Inactivation of lung surfactant by a variety of mechanisms has been proposed to contribute to the pathology of acute Respiratory Distress Syndrome (ARDS) [1], respiratory syncytial virus bronchiolitis in infants [2], and respiratory infections and cystic fibrosis [3,4].

In addition to opposing surface tension forces within the alveolus, surfactant has also been reported to promote mucociliary clearance [5], bind aeroallergens, pollens, bacteria and other particulates [6], oppose airway oedema [7] and prevent collapse of terminal airways [8]. The phospholipid component of surfactant will inhibit both proliferation of peripheral blood lymphocytes [9,10] and the neutrophil respiratory burst oxidase response [11], and is thought to act generally as an immunosuppressive agent in the lungs. In contrast, the water soluble surfactant proteins A (SP-A) and D (SP-D) bind and aggregate gram negative bacteria, act as opsonizing agents, and stimulate alveolar macrophage and neutrophil inflammatory responses [12].

The paper by Hills and Chen in this issue highlights another proposed role for airway phospholipids in normal lung function that may become compromised in asthmatic patients [13]. They have previously presented microscopic evidence that multiple layers of phospholipid bind directly to the surface of the bronchial epithelium [for review see 14], and have suggested that such layers may contribute a barrier function at the epithelial surface. In their present paper they have administered a dry powder mixture of phospholipid to the lungs of ventilated rats, and report that this reduces the amplitude of neurological response to stretch receptors. They go on to hypothesize that, if this phospholipid barrier was eroded as part of the disease process in asthma, then it might contribute to the sensitivity and overall magnitude of the bronchoconstriction response. Finally, they use this suggestion to argue that administration of phospholipid mixtures may provide clinical benefit in asthma.

These suggestions are intriguing and deserve a full examination. There are three especially relevant questions in this context. First, what are the other proposed functions of lung surfactant in the pathology of asthma? Second, what is the relationship between epithelial bound phospholipid and lung surfactant? Third, what are the possibilities for surfactant therapy in asthma?

Lung surfactant, airway function and asthma

Lung surfactant is a complex mixture of lipid and protein defined essentially as the material that can be purified from a saline lavage of the lungs that will generate near zero surface tension values at the air–liquid interface when it is laterally compressed in a surface tension balance. However, this functional definition is based on measurements *in vitro* and does not directly address the contentious debate about how surfactant acts within the alveolus. Functional surfactant is conventionally envisaged as a monomeric or multimeric layer at the air–liquid interface. According to the equation of La Place, in the absence of surfactant the high surface tension of water would cause the collapsing pressure of the alveolus to increase as alveolar radius decreases when breathing out. In this model of surfactant action, alveolar contraction would compress the surfactant lining layer, decrease surface tension further and prevent lung collapse. Bangham has forcibly pointed out that the concept of zero surface tension at body temperature is impossible [15], and claims that the conventional view of surfactant action is an artefact of the analytical method used. Instead, he proposes that surfactant exists as rafts of saturated phospholipid that are solid at body temperatures, and which exert a lateral physical force that splint open alveoli. These surfactant rafts would then crack in response to alveolar expansion on inhalation, allowing adsorption and desorption of surfactant components to and from the air–liquid interface. Hills has taken Bangham's concepts further and proposes that the alveolus is essentially dry with little true air–liquid interface. In his model, surfactant phospholipid is envisaged as binding directly to the alveolar epithelial surface, with pockets of epithelial lining fluid restricted to interstices and corners between cells [14].

While a better understanding of the mechanism of surfactant action in the alveolus is important for studies of surfactant turnover, metabolism and function, it is probably not immediately relevant to clinical practice. Whatever its precise mode of action, surfactant in effect minimizes surface tension forces within the alveolus and permits normal

breathing. The situation within the airways is much less clear. The precise role of airway surfactant has yet to be fully established, its distribution pattern between the various components of the airway lining fluid is not clear, and the requirements for surfactant function may vary at different levels of the bronchial tree. Surfactant can be isolated by saline washing at all parts of the porcine airways; such material purified by conventional density gradient centrifugation has a composition comparable to surfactant purified from bronchoalveolar lavage fluid (BALF). Analysis of phospholipid compositions of the bronchial mucosa showed it to be very different from that of both airway and alveolar surfactant [16]. This observation supports the concept that airway surfactant is derived from alveolar sources, and is transported from distal to proximal parts of the lungs by a combination of surface pressure and mucociliary action. Importantly, there is no published evidence for secretion of intact functional surfactant from the bronchial epithelium. Clara cells, for instance, express and synthesize SP-A, SP-B and SP-C, but do not accumulate or secrete lamellar bodies, or synthesize the disaturated phosphatidylcholine (PC) species characteristic of surfactant.

Major functions proposed for surfactant in the airways are to maintain patency of terminal and conducting airways and to prevent liquid and mucus plugging. It is envisaged that high surface pressures generated by surfactant in effect squeeze liquid out of the airway and provide a physical force to oppose airway collapse. This concept is supported indirectly by both animal and clinical studies. Administration of exogenous surfactant to the lungs of sensitized guinea-pigs significantly diminished allergen-induced bronchoconstriction [17,18], and a beneficial effect surfactant therapy has been reported anecdotally in acute asthma [19]. A variety of plasma proteins, especially fibrin monomer, inhibit the surface tension reducing properties of purified surfactant *in vitro* [20]. It is suggested that leakage of protein-rich exudate into the airways as part of the initial inflammatory response in asthma inhibits airway surfactant function, permitting greater influx of liquid. This would then lead to the establishment of a vicious cycle and, eventually, the liquid plugging of conducting airways characteristic of asthma. Experimental evidence to support this concept has recently been provided by two studies showing inhibition of surfactant function after local allergen challenge to patients with mild asthma [21,22].

Epithelial-bound phospholipid

All these models of surfactant function in the airways visualize the existence of a surface active phospholipid layer at the air-liquid interface, a concept supported by electron microscopy examination [23]. In contrast, the phospholipid layer described by Hills and Chen is bound

directly to the bronchial epithelium and consequently, by definition, below the mucus layer in the airways. The existence of such a phospholipid barrier would not be surprising, as comparable bound phospholipid layers are found at most epithelial surfaces studied including gastric mucosa [24] and pleural epithelium [25]. The possibility that such a barrier in the airways may serve to mask irritant receptors is an interesting concept, may prove relevant to the pathology of asthma and certainly merits further investigation. However the relationship between this material and conventional surfactant isolated from BALF or sputum samples is not clear. As the bound phospholipid would not be removed by saline lavage, its composition has never been determined directly. Indirect evidence, however, suggests that purified surfactant phospholipid and bound phospholipid may have very different compositions [16]. Surfactant purified from saline lavage of pig bronchi had a composition characteristic of alveolar surfactant, with high concentrations of the active components dipalmitoyl PC and phosphatidylglycerol (PG). Both these surfactant components were present at low concentrations in lipid extracts from the underlying bronchial epithelium, which presumably still contained the bound phospholipid layer. Consequently, the most probable model to emerge is the existence of two distinct phospholipid layers with different spatial locations and different compositions and function.

There are good reasons for the bronchial epithelial bound phospholipid layer not to have the composition of alveolar surfactant. Surfactant from amphibia, which have poorly developed lungs lacking alveoli, contains low concentrations of dipalmitoyl PC and this is consistent with it acting as an 'antiglu' factor [26]. In evolutionary terms, it was the development of small alveoli essential for efficient breathing in the mammalian lung that was associated with an increased concentration of dipalmitoyl PC in surfactant to combat the resultant high surface tension forces. As the bound phospholipid layer would not be located at the air-liquid interface in the airways, there would be no reason for it to have a high content of dipalmitoyl PC. This situation is directly comparable to that in the stomach, where a phospholipid lining layer has been demonstrated which protects the gastric mucosa from back-diffusion of hydrogen ions [24]. Although often termed a gastric surfactant by analogy with lung surfactant, direct compositional analysis of this material has shown unequivocally that it contains negligible dipalmitoyl PC or PG [27]. As with the phospholipid layer bound to the bronchial epithelium, the gastric mucosal phospholipid barrier is not located at an air-liquid interface and consequently does not have the composition of lung surfactant.

Surfactant therapy in asthma

One problem in understanding the relevance of the use of

exogenous surfactant in Hills and Chen's paper is a lack of definition of terminologies. All phospholipids contain hydrophobic fatty acyl and hydrophilic charged regions and consequently are all surface active, but to differing extents. Consequently, the term surface active phospholipid (SAPL) used in this paper could be misleading; it does not necessarily equate with active lung surfactant. While giving an exogenous phospholipid surfactant based on dipalmitoyl PC subdued the neurological response to metacholine, this does not imply that the endogenous phospholipid barrier in the airways has a similar composition. Moreover, the relative importance of vagal stimulation to the extent of the acute asthmatic response is debatable, and Hills and Chen have not yet addressed any role of any barrier phospholipid layer in other major contributory factors in the allergic asthmatic response.

While all available evidence suggests that there is no absolute deficiency of surfactant phospholipid in asthmatic patients, it is possible that there may be a more specific erosion of the bound phospholipid barrier in the airways. This possibility has not yet been tested. Quantification of surfactant concentration from samples obtained by bronchoscopy or sputum induction is inevitably problematic due to variations in dilution factors and recovery [28], but it appears that the basic surfactant dysfunction in asthma is inhibition of surface activity by the protein component of the plasma infiltrate [20]. The major goal for surfactant therapy in asthma must be to provide sufficient exogenous surfactant to overcome this inhibition. Compared with the large amounts of surfactant given to preterm infants at risk of RDS (relative to body mass), amounts of surfactant required to treat the airways rather than the whole lung parenchyma in asthma will probably be relatively modest. The optimal composition of surfactant to be administered, however, is far from clear. The dry powder device described in the paper by Hills and Chen is a novel and potential exciting delivery system. Surfactant phospholipid in the anhydrous form, instead of being reconstituted in a saline suspension, spreads rapidly over a liquid surface [29] and theoretical considerations suggests that this dry powder surfactant mist will target the smaller airways and alveolar regions. Whether such a surfactant should also ideally contain one or more of the surfactant proteins is uncertain. SP-B and SP-C are essential for endogenous surfactant function, and act to promote adsorption of hydrated phospholipid to the interface layer in the alveolus, whether that is compressed monolayer, solid sheet or bound phospholipid. It is not yet known whether they will enhance the activity of dry surfactant phospholipid given to the surface of the lungs. Any requirement for SP-A or SP-D is even more uncertain, as neither of these proteins has been included in the formulation of any commercially available therapeutic surfactant. However, studies *in vitro* have suggested that SP-A

enhances resistance of surfactant phospholipid to functional inhibition by plasma proteins.

There are a number of additional considerations for the design of therapeutic surfactants in asthma. In addition to their physical effects on lung function, surfactant phospholipids, especially PC and PG, are also potent regulators of inflammatory cell function in the lungs. They inhibit T-lymphocyte proliferation, neutrophil respiratory burst oxidase and cytokine production by alveolar macrophages. Any formulation of an active surfactant for treating asthma must take into account its overall effects on the inflammatory cell response. Similarly, SP-A and SP-D are integral components of the innate defence system of the lungs. While SP-D is apparently essentially antimicrobial in its actions, SP-A in addition to acting as an opsonizing agent also binds pollens and other aeroallergens such as the derP1 antigen from the house dust mite. Moreover, binding of derP1 to SP-A prevents its subsequent binding to specific IgE. However, as the actions of surfactant phospholipids compete with and oppose those of SP-A and SP-D, the role of surfactant function in asthma is becoming increasingly complicated. The work presented here by Hills and Chen provides yet another level of complexity. There are currently a number of ongoing trials of surfactant therapy in asthma, but caution will have to be exercised in interpretation of their outcomes. Lack of response may well be due either to using surfactant of less than optimal composition or to giving it by an inefficient delivery system.

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