Review

Highlights of Early Pulmonary Surfactant:
Research from Bench to Clinic

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Abbreviations
FDA= Food and Drug Administration
NIH= National Institutes of Health
DPPC= dipalmitoyl phosphatidylcholine
SP-= surfactant proteins
BAL= bronchoalveolar lavage
RDS= respiratory distress syndrome
SP-A= surfactant protein A
AMs= alveolar macrophages
UTR= untranslated region

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SUMMARY. Nowadays we know a great deal about the lung. We understand its major functions, how it achieves most of these, how it looks microscopically, and other physiological attributes such as that adequate amounts of pulmonary surfactant in the prematurely born infant are essential for lung function and consequently for life. In this review, we summarize highlights of the history, i.e. the journey of pulmonary surfactant discovery and how it moved from the lab bench to the patient's bedside. *Pneumon 2013, 26(4):350-354.*

INTRODUCTION

The surfactant journey spans decades of research starting in the 1920's. In the early 1990's the first surfactant preparation was approved by the Food and Drug Administration (FDA) for clinical use in the prematurely born infants. The National Institutes of Health (NIH) considered the surfactant replacement therapy a success story. Indeed, the surfactant replacement therapy has resulted in saving many lives of the prematurely born infants world-wide as well as in reducing the morbidity of such babies. In this brief account of the history of surfactant discovery and its transition to the bedside, we highlight the fact that although surfactant replacement therapy has been considered a success story and now is routinely used in the clinic, the initial steps were challenging.

The key functions of the lung include its ability to carry out the essential for life function, the O_2/CO_2 exchange, and defend itself (and the body) from harmful agents that the lung is exposed to during respiration. Pulmonary surfactant is of paramount importance for life because it prevents collapse of the distal airspaces or alveoli at low lung volumes and thus enables the lung to carry out the O_2/CO_2 exchange.

Pulmonary surfactant is a lipoprotein complex that consists of several different types of lipids with dipalmitoyl phosphatidylcholine (DPPC) being the major saturated phospholipid, as well as proteins that include both hydrophobic and hydrophilic components. Four surfactant proteins (SP-) A, B, C, and D have been shown to play important roles in surfactant-related functions and/or innate host defense. The initial clinical surfactant replacement studies were short of success in part due to the choice of the

surfactant component chosen for treatment of prematurely born infants. DPPC, which is the major phospholipid component of pulmonary surfactant, was initially used in surfactant replacement studies. These studies were met with failure because the phase-transition temperature of DPPC is above 37 ºC and therefore at body temperature being at its solid state could not spread.

Today there are several surfactant preparations used in the clinic or in clinical trials[1]. Most of these are derived from a natural source such as bovine[2] or porcine lung[3] and/or bronchoalveolar lavage (BAL). Synthetic surfactants have also been tried but have been proved to be less efficient than the natural preparations[4]. In the foreseeable future, as our knowledge of the function of surfactant components, alone or in combination, increases, it is possible that better surfactant preparations will reach the clinic that may be condition- or disease- specific.

THE EARLY PERIOD (1920's – 1950's)

The distinctive characteristic of the lung to expand and retract was recognized early on and the expansion/contraction property of the lung was initially attributed to its elasticity. In fact, the lung was seen as a plain bag for gas exchange[5] and no one credited the lung with having an active metabolic life that would include production of a substance such as the surfactant.

Dr. Kurt von Neergaard, a Swiss physiologist in the **1920's** brought forward the idea that a number of forces play a role in the expansion and retraction of the lung[6]. Given the fact that the terminal airspaces are spherical like structures, he proposed that these structures are subject to forces as dictated by the law of Young and Laplace ($P = \frac{4T}{r}$). However, he also realized that according to this law, the pressure exerted on a spherical-like structure, such as the alveolus, increases, as the radius decreases, and this would result in the collapse of the small alveoli. Because this does not happen in the normal mature lung, Neergaard hypothesized that there must be a substance, yet to be identified, that lowers the surface tension preventing thus alveolar collapse. Hence, he did not only recognize the importance of forces and specifically that of surface tension in lung expansion and contraction, but also conceptually recognized the existence of "a substance" that would be critical in preventing alveolar collapse. The proposed substance was later identified as pulmonary surfactant.

In **1947** Dr. Peter Gruenwald observed that lower pressure is needed to fill the lungs of newborn babies (that had passed away for different reasons) with a salt solution than with air. He attributed this to the principles of surface tension and he experimented with "surface active substances" that lowered the surface tension[7]. Unable to make the clinical connection he stopped his inquiry there.

Nearly a quarter of a century went by before Neergaard's vision of the existence of surfactant was revisited. This delay was due, in part, to the fact that the focus was on the presence of "hyaline membranes", which were thought to be the cause of respiratory problems in prematurely born babies. These membranes were thought to form when babies breathed in amniotic fluid. As we now know, the hyaline membranes are the result rather than the cause of respiratory distress.

In **1955** the British physicist Richard Pattle observed that a "foamy" substance lines the surface of alveoli and that the bubbles from this substance were stable for 1 h or even longer, compared to bubbles from other fluids, such as blood, that lasted only a few minutes, indicating that the surface tension in the lung was low. He surmised that the bubbles must be covered with a unique substance that confers the observed stability. He published his scientific findings in Nature in 1955[8]. In 1958 he even noted that the absence of a lung lining substance may sometimes be one of the difficulties with which a prematurely born baby has to contend with and may possibly play a role in the failure of lungs to expand at birth[9].

Almost in parallel Dr. John Clements who was a volunteer for military service, was assigned the task of figuring out the disastrous effects of war gases on the lung tissue. Rather than focusing on the entire lung, or on lung extracts, he focused on the bubbles that appeared in the airways of the exposed lungs[10,11]. Dr Clements, a physiologist, along with the Canadian pathologist Chris Macklin developed ways to measure surface tension[11,12]. They observed that the surface tension of this yet unknown foamy material was low and that it varied according to the surface area and the function of the lung (i.e. inhalation and exhalation). When the material was compressed it reached a surface tension of <10 dynes/cm and when stretched out it was >45 dynes/cm. It is noteworthy to mention that the Pattle, and the Clements - Macklin teams came to the same conclusions independently and within a few months of each other. Dr. Clements initially named this substance "anti-atelectasis factor" (atelectasis is defined as the incomplete expansion), and later named it pulmonary surfactant[11]. He also highlighted the essential role of surfactant in its ability to maintain a healthy lung.

Notably, surfactant can prevent at low lung volumes alveolar collapse caused by large lung pressure and at high lung volumes can prevent overexpansion of the lung by hindering the surface tension to rise, enabling alveoli of different size to function with equal efficiency.

Another very important contribution in the 1950's was from pediatrician Dr. Mary Ellen Avery. She observed that the lungs of babies that died from respiratory distress syndrome (RDS) were more liver-like (i.e. lacked air) rather than normal lung-like and that these lungs lacked the foamy substance that Clements named it "pulmonary surfactant"[13]. Her experiments demonstrated that the surface tension of the compressed lung extracts from babies who died from RDS remained high, 20-30 dynes/cm, as opposed to the surface tension of compressed lung extracts from individuals that demised from other causes, which was low, 5-10 dynes/cm. She then postulated that the high surface tension in the non-foamy lungs causes alveolar collapse. She also asserted that lack of surfactant could associate with prematurity indicating that babies born prematurely may have not had the chance to produce surfactant. With her work, she was able to make the clinical connection of the lack of surfactant with RDS and to promote the notion of surfactant production as a developmental process. In **1959**, Dr. Avery and her colleague, Dr. Jere Mead, published their important findings in American Journal of Diseases of Childhood[13], putting to rest the hypothesis that hyaline membranes caused RDS.

The 1950's was indisputably the decade that started the field of the pulmonary surfactant. Pattle and Clements – Macklin team discovered the foamy substance in the lung that Neergaard speculated its existence in the 1920's, described the characteristics of the surface tension, and also named the foamy substance surfactant. Dr. Avery on the other hand correlated pulmonary surfactant to a clinical problem.

The surfactant period from 1960's – 1980's is the period where studies were carried out regarding the potential use of surfactant in the clinic and the initial characterization of surfactant components.

In **1972** a surfactant replacement experiment was carried out in animals. Drs. Enhorning and Robertson in Sweden treated prematurely delivered rabbits with surfactant obtained from adult rabbits and observed that the lungs of the treated prematurely delivered pups were well aerated and the pups did not die as expected[14,15]. In the same year the biochemical composition of surfactant was identified by Drs King and Clements[16-18]. Their study revealed that surfactant is a lipoprotein complex consisting of several types of lipids (80% phospholipids, 8% neutral lipids such as fatty acids and cholesterol) and proteins (12%)[19]. About 80% of the phospholipids were found to be phosphatidylcholine, and most of the phosphatidylcholine to be DPPC. The phosphatidylglycerol is the second most abundant phosholipid in surfactant and in the course of lung development is found in an inverse proportion with phosphatidylinositol. In **1977** Adams and colleagues showed that natural bovine surfactant has beneficial effects on prematurely born lambs[20].

Although, the use of surfactant in clinical trials began in the 1970's, the results were initially negative. DPPC used instead of a naturally derived surfactant[21]. DPPC at body temperature is at a solid state because its phase transition temperature is at 41°C. Thus at body temperature and in the absence of other surfactant components, DPPC cannot spread on the surface of the alveoli.

However, in **1980** Dr. Fujiwara in Japan, encouraged by the findings from the animal studies where natural surfactant was used, carried out the first promising clinical trial using surfactant from bovine lung extracts[22]. This consequently led to several clinical trials where many different natural and synthetic surfactant preparations were assessed in the treatment and/or prevention of RDS in neonates[23]. The clinical trials involved the following categories: i) synthetic or protein free preparations, ii) natural minced lung extracts, iii) natural lung lavage extracts, v) natural amniotic fluid extracts, and vi) synthetic protein analogs[24]. In the early **1990's**, the FDA approved surfactant replacement therapy for clinical use in the prematurely born infants. The National Institutes of Health (NIH) considered the surfactant replacement therapy for the prematurely born infants "a success story". Since then several types of surfactant have come to the market that include bovine and pig derived surfactants, and may differ not only with regards to the species origin (bovine, porcine), but also in the method of preparation and/or composition[25].

COMMENTS

Surfactant replacement therapy is indeed a success story and has helped significantly in reducing the mortality and morbidity of the prematurely born infants suffering from RDS but has not eliminated the disease. Thus, research continues. A great deal of work has been carried out and continues to go on about the composition of surfactant used in the clinic and the function of its various components. For example, the surfactant protein

A (SP-A), a multifunctional collectin protein, plays an important role not only in the surfactant structure and proper function but also in lung innate host defense including pathogen phagocytosis and cytokine production through the alveolar macrophages (AMs)[26-32]. SP-A in humans is encoded by two homologous genes SP-A1 and SP-A2, each having several splice and sequence variants[33,34]. The 5'untranslated region (UTR) splice variants and the 3'UTR sequence variants have been shown to play a role in their regulation[35-38]. The function of SP-A1 and SP-A2 has also been shown to differ[33,39-44] and both gene products are necessary for the extracellular structural form of surfactant called tubular myelin[45]. Moreover, an imbalanced expression of SP-A1 and SP-A2 has been shown to correlate with increased lung disease risk[46,47], pointing to a need for a better understanding of the regulatory mechanisms. A better understanding of the regulation of SP-A1 and SP-A2 may help provide points of intervention to enhance gene specific expression under various conditions.

Despite the fact that new information and cutting edge technology enabled modern surfactant replacements to become closer to naturally occurring surfactant, the commercially available surfactants lack innate host defense surfactant proteins (SP-A and SP-D). These proteins are critical for lung homeostasis, surfactant metabolism, innate and adaptive lung immunity[48]. In neonatal RDS, the prematurely born infants have significantly decreased levels of these surfactant defense proteins compared to full term infants. Given the fact that a major complication in the prematurely born infant is infection, inclusion of these proteins in future surfactant preparations is a must. Alternatively, these proteins may also be used by themselves for therapy of derangement of innate immunity. In fact an SP-A rescue animal study demonstrates that the latter scenario is feasible, therefore providing support for therapeutic use of SP-A[49]. In that study SP-A -/- mice were treated with a single dose of human SP-A, and at different times after treatment the AMs were isolated and their expression proteomic profile was analyzed. The proteomic profile of the treated AMs approximated that of the wild type mice and was very different from AMs derived from the SP-A -/- mice.

Furthermore, genetic variants of surfactant proteins, either single nucleotide polymorphisms, insertion/deletion variants, or haplotypes have been shown to correlate with lung inflammatory processes, surfactant derangement and several lung diseases[34,50]. Findings from ongoing and future research of the SP-A variants and their role in innate host defense and surfactant-related functions may be useful in consideration of personalized medical regimens. For example, should we understand the mechanisms of differential expression of SP-A variants, we will be able to modulate expression depending on the particular environmental stressor, SP-A variant, and/or functional activity of surfactant.

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