The era of pulmonary surfactant from Laplace to nowadays

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Abstract. The first observations on neonatal respiratory distress syndrome (RDS) were published by some obstetricians in England, France and Germany in the second half of the 18th century. The concept that RDS might involve the absence of something stems from the observations of a Swiss physiologist, Kurt von Neergaard, who published an article in 1929 about a fundamental principle of respiratory mechanics: the surface tension in the alveoli. Further early descriptions of the existence, composition, and synthesis of the surfactant complex and its physiologic role in maintaining alveolar stability were dependent on the pioneering contributions of Radford, Macklin, Pattle, and Clements (among others). But the final link, describing surfactant deficiency as a cause of RDS, came from Avery and Mead in 1959, when they showed lung extracts from babies with hyaline membrane disease deficient in surfactant. Understanding surfactant composition, function and therapeutic usefulness has increased exponentially over the last 50 years and this paper reorganizes the steps of the research in this field until nowadays. Most of the discussion concerns the fundamental role of lung surfactant in RDS of premature infants, and the success of exogenous surfactant replacement in the clinical therapy of this disease.

Key words: preterm infants, respiratory distress syndrome, pulmonary surfactant, surface-tension

Introduction

The discovery of the cause and of the possible pharmacological treatment of the respiratory distress syndrome (RDS) in preterm infants has not been without obstacles. Anyhow, the basis of the actual knowledge is the physical law exposed in 1806 by the physicist and astronomer Pierre Simon de Laplace. In fact, the fourth volume of his "Traité de Mécanique Céleste" includes a chapter on the function of blood capillaries, in which he reported the mathematical relation among pressure, surface tension and radius of the bending surface useful to understand the action of surfactant on the alveolar wall (Laplace's law) (1).

We will divide the history of the scientific discoveries on pulmonary surfactant into three main paragraphs corresponding to three different ways of research development (Table 1).

Clinical proofs of RDS and first of treatment attempts

In 1835 a German obstetrician, Eduard Jorg, published a large monography on RDS containing several conceptually modern articles concerning the disease: he explained that it developed almost exclusively in the preterm infants and that asphyxia at birth, maternal diseases, hypothermia and loss of blood for detachment of placenta might contribute to its pathogenesis. The most frequent complications, as one can read in his book, were represented by persistent right-to-left shunt through a patent ductus arteriosus, pneumothorax, pulmonary interstitial emphysema and pneumonia. Besides, he stated that body massage, heating, artificial ventilation and oxygen administration (but at low concentration because he had found inflammation of the small airways giving oxygen at high concentrations) might be valid treatments for this neonatal disease (2).

Table 1. Chronology of researches on surfactant

Year interval	Clinical proofs of RDS and first attempts of treatment	Scientific proofs of surface active agent	Experimental and clinical application of surface active agent
1835-1947	• Monography on RDS (2) • Project a respirator for infants (3) • Device for long-term mechanical ventilation (4) • First results on mechanical ventilation in infants (5)	• Shows presence of alveolar tensioactive substances (7) • Lung contains high amounts of DPPC (9) • States that the resistance to aeration is due to surface tension (8)	
1954-1970		• Presence of an intraalveolar film maintaining a constant favourable surface tension (10) • Hyaline membranes are the cause of clinical RDS (11) • RDS is due to lung surfactant deficiency (12) • Extraction of a phospholipid fraction from bovine lungs with tense active properties (13) • Aerosolized DPPC did not work on RDS (14) • DPPC produced and secreted into amniotic fluid (15) • Steroids improved fetal lung maturation in animals (17)	• Use of surfactant extracts improves the pulmonary mechanics in premature lambs (28)
1971-1977	• Endotracheal continuous positive airway pressure (6)	• Lecithin/sphingomyelin test to assess lung maturation before birth (18) • Isolation of surfactant apoproteins (21) • Surfactant is stored in type II cells' lamellar bodies (23) • PG is absent in RDS (20) • Lamellar bodies convert into tubular myelin (24) • Infusion of plasminogen can decrease severity of RDS in clinical trial (25)	• Use of surfactant extracts improves the pulmonary mechanics in premature rabbit pups (26)
1980-2001			• "Recipe" for the exogenous natural surfactant (29) • Exogenous human surfactant for RDS treatment (32) • Develop of artificial protein-free surfactants (35) • Isolation and characterization of the human pulmonary surfactant apoprotein genes (44) • Biophysical mechanism by which plasma proteins inhibit lung surfactant activity (49) • Efficacy of porcine natural surfactant also in moderately severe and serious neonatal RDS (39,40) • Prophylaxis of RDS by treatment with modified porcine surfactant at birth (41) • Clinical trials on efficacy and safety of semi-synthetic surfactants in RDS (46) • Protein leakage in the alveolar spaces (which has surfactant inhibiting properties) occurs in the further course of RDS (47,48,49,50,51) • Clinical trials on differences in the sensitivity of various surfactant preparations to the inhibitory capacities of leaked proteins (53,54,55,58,59).

Legend: RDS = respiratory distress syndrome; DPPC = dipalmitoylphosphatidylcholine; PG = phosphatidylglycerol

In 1889 Alexander Graham Bell, the Canadian inventor of the telephone, projected a respirator, sized to the newborn infant, that surrounded the body (3). Indeed, he stated that many preterm infants died because of failure to expand sufficiently their lungs at their first breath and that it would have been possible to save their life starting artificial ventilation. Anyway, the respirator invented by Bell remained substantially unused and the preterm infants went on dying at least till to 1929 when Drinker and Shaw developed an equipment for long-term mechanical ventilation (4). However, artificial ventilation as a treatment for RDS started to be accepted only after the article published in 1953 by Donald and Lord (5). In spite of this it needed 15 years before artificial ventilation became the specific treatment for RDS. In particular, the application of the invention of George Gregory, an anesthesiologist working together with a group of neonatologists in San Francisco, allowed to reduce mortality due to RDS to about 20%; in fact he built a ventilation system with air under continuous positive pressure administered through the endotracheal tube, able to improve the alveoli opening at the end of exhalation (6).

Scientific proofs of a surface active agent (surfactant)

Already in 1929 the studies of Kurt von Neergard, a Swedish physiologist working in Switzerland in Basilea, had shown that the presence of alveolar tensioactive substances is necessary to maintain a regular pulmonary ventilation, and, thanks to the measurement of the pressure-volume curves of the lung of excised laboratory animals (dogs and pigs), he evidenced that in whatever state of lung expansion, the surface tension was responsible for the total retraction of the lung much more than the only elasticity of the lung tissue (7).

In 1947 the pathologist Peter Gruenwald, without knowing the von Neergaard's work, had carried out a set of experiments that induced him to state that "the resistance to aeration is due to surface tension which counteracts the entrance of air but has no effect on the aspiration of fluid. Surface active substances reduce the pressure necessary for aeration" (8). He speculated that the addition of surface active substances to the air or oxygen-air blend, both spontaneously inhaled and introduced by a respirator, might aid to relieve the initial atelectasis of newborn infants.

In 1946, Thannhauser had shown that the lung contains unusually high amounts of dipalmitoylphosphatidylcholine or DPPC (9), but he did not connect this finding with that of a surface active substance inside the alveolus.

Macklin, a Canadian researcher, in 1954, published a paper in which he described the presence of an aqueous mucopolysaccharide film on the pulmonary alveolar walls which is able to maintain "a constant favourable surface tension" (10). In the same paper he also stated that such a film is originated and secreted from the granular pneumonocytes (10).

In 1956, Gitlin and Craig published a study which connected hyaline membranes formation with birth asphyxia and theorized that they were the cause of the clinical distress of RDS, in contrast with the current theories that considered the hyaline membrane the terminal product rather than the cause of the clinical respiratory distress. This study also showed that hyaline membranes are mainly composed of fibrin (11). They speculated that the pulmonary hyaline membranes can be produced as the result of two phenomena: 1) interstitial essudate formation by effusion from pulmonary capillaries and 2) conversion of leaked fibrinogen into fibrin. The theory that hyaline membranes resulted from fibrinolysin deficiency or disseminated intravascular coagulation, has lasted for many years and has stimulated several therapeutic approaches directed to influence coagulation.

Eventually, in the paper published in 1959, Mary Ellen Avery and Jere Mead showed that the newborn infants, who died of hyaline membrane disease (HMD), had high pulmonary surface tension. They concluded that the disease was associated with the absence of a tensioactive substance (surface active agent = surfactant) able to lower surface tension when alveolar volume is decreased (12). In 1961, Marshall Klaus and colleagues isolated the alveolar surfactant from bovine lungs and extracted a phospholipid fraction that displayed itself a surface active behaviour (13).

Avery and Mead's discoveries, together with identification of DPPC as the major surfactant com-

ponent, induced many neonatologists to employ DPPC, usually in nebulized form, in several clinical trials on RDS treatment. However a study directed by Clements and performed in Singapore in 1967 by Chu et al. (14), showed that aerosolized DPPC was uneffective and might worsen the clinical course of RDS. It was then supposed that components other than only DPPC, have to be administered to achieve optimal reduction of surface tension at the alveolar air-fluid interface, and therefore to decrease severity of RDS.

In the same year, Louise Gluck et al. had shown that during the development of the mammalian lung, DPPC is produced and secreted into the amniotic fluid (15) and, in 1972, they developed the first useful test to determine fetal lung maturity by using the measurement of the lecithin/sphigomyelin ratio in amniotic fluid (16). This test, still clinically used to programme some premature deliveries at high risk for lung immaturity, evaluates the relative presence of the two substances and gives lung maturity for values greater than 2.

In 1968, in New Zealand, the obstetrician Graham Liggins noted that, following infusion of adrenocorticotropic hormone, cortisol, or dexamethasone to the mother, premature lambs had unexpectedly mature lungs (17). Liggins carried out a controlled trial of betamethasone which proved that steroid administration reduced morbidity and mortality from RDS in premature infants (18). However, RDS remained responsible for a quarter of all neonatal deaths at least until 1973 (19).

A Finnish neonatologist, Mikko Hallman, in 1975, reported that phosphatidylglycerol (PG) contributed to surfactant spreading, and that this lipid is invariably absent in RDS (20). In a series of papers published in 1972 and 1973, Richard King showed that surfactant also contains several specific apoproteins (21,22), and that the greatest surfactant protein, SP-A, interacts with phospholipids and is responsible for the interaction of surfactant with the cell surface.

Gil and Reiss identified the lamellar bodies of type II cell as the site of intracellular surfactant storage (23), and Mary Williams demonstrated the intraalveolar transformation of lamellar bodies into tubular myelin (24), a particular form of pre-active surfactant.

In 1977 Ambrus et al. published the results of a double-blind trial of 500 preterm infants (25) showing that infants with RDS had low levels of serum plasminogen and that plasminogen infusion was able to decrease the severity of the disease and the overall neonatal mortality: therapeutic administration of plasminogen enhanced fibrin degradation in the alveoli with subsequently improved alveolar expansion. The "hyaline membranes", in fact, consist mainly of fibrin and it is true that premature infants present a relative lack of serum plasminogen (which in turn leaves them defenseless against pulmonary fibrin deposition) but it was not possible to use plasminogen as a preventive agent in RDS because pulmonary fibrin deposition and subsequently hyaline membranes formation are end products rather than causes of RDS (which, as Avery and Mead had shown, is due to surfactant deficiency). So, later on, plasminogen administration was neglected in clinical trials.

Experimental and clinical applications of the surface active agents concepts

In 1972, Enhorning and Robertson started to use surfactant extracts from adult rabbits to treat prematurely born rabbit pups and showed significant improvement in pulmonary mechanics (26,27). Similar studies were performed by Forrest Adams et al. in premature lambs (28). From their work Tetsuro Fujiwara derived the "recipe" for the exogenous natural surfactant that was found effective in the treatment of newborn infants affected by RDS (29). This surfactant was isolated from minced bovine lung and enriched with synthetic lipids. Within 3 hours from endotracheal application, paO_2 rose from a mean of 45 mmHg to 212 mmHg and supplementary oxygen could be lowered from 81% to 38%. After that study, several effective natural surfactants were prepared from calf lung lavage, minced bovine and porcine lung and from human amniotic fluid (30, 31, 32, 33). In order to overcome the problems of potentially immunogenic foreign proteins, of viral contamination, and the shortage in material, synthetic surfactants were developed too (34, 35). Synthetic surfactants are nowadays rarely used because they are significantly less effective

than natural surfactants (36, 37, 38). Surfactants obtained from human amniotic fluid were early abandoned early in the clinical practice because they were too laborious to be prepared. In fact to obtain 1 dose of surfactant amniotic fluid from 3 pregnancies is necessary and moreover they do not preserve from immunological reactions and their sterility is difficult to be guaranteed.

At present several thousands infants have been treated with available marketed surfactants, mainly bovine and porcine, and efficacy of exogenous surfactant on mortality and severity of RDS is proved.

In our experience with porcine surfactant we observed significant reduction of mortality, pneumothorax and oxygen requirements already few minutes after the surfactant administration in the severe form of RDS (39). This effect was confirmed by earlier use, i.e. in less advanced grade of RDS, allowing moreover to stop its evolution towards a more advanced grade (40). Also, the use of surfactant as prophylaxis of the RDS in the babies of gestational age less than 30 weeks was effective in reducing both mortality and development of the disease (41, 42, 43).

However, research does not stop here and, since the genes encoding for the surfactant proteins have been characterized (44, 45), some semi-synthetic surfactants, which contain apo-proteins synthesized in laboratory, are now in phase of study (46). The first results from these studies are so far not encouraging and many technical problems have to be solved. However this seems the way for the future, not only to reduce possible compatibility problems, but also to produce low-price surfactant to be used also for diseases other than RDS that consume or lack surfactant as meconium aspiration syndrome, infections, pulmonary hypoplasia, adult RDS of the newborn, congenital defect of surfactant protein B, and possibly asthma, or also to deliver drugs into the lung.

Another important knowledge about pathogenesis of RDS is that, in addition to the primary lack of surfactant, substantial protein leakage in the alveolar spaces occurs in the further course of RDS, which may significantly contribute to damage surfactant function (47). Several *in vitro* studies have demonstrated surfactant inhibiting properties of albumin (48), haemoglobin (49) and, overall, fibrinogen and fibrin monomer (50). These proteins may inhibit surfactant function by preventing the surfactant phospholipids from adsorbing to the air-liquid interface, possibly by a competition for space at the air-liquid interface between the proteins and surfactant phospholipids rather than by direct molecular interactions between proteins and surfactant (51). From these observations derives the importance of timing of exogenous surfactant administration (preventive *versus* rescue administration or, in other words, preceding leakage of plasma proteins into the alveolar space) in preterm infants to improve replacement therapy efficacy.

Several studies (31, 52, 53, 54, 55) have demonstrated that the various surfactant preparations show different sensitivity to the inhibitory properties of leaked proteins depending on different lipid and apoprotein composition and on presence of contaminating materials. These differences may have an impact on the surfactant function when increased alveolar protein load is present, such as late RDS and adult RDS. An important role in the determining surfactant resistance to the serum proteins in an injured lung is carried out by surfactant-associated proteins SP-B and SP-C (56). In fact studies comparing synthetic to natural surfactant (57) have shown that surfactant proteins B and C (which are only present in natural surfactant) are needed to enhance rapid adsorption and spreading of phospholipids and to resist to plasma protein inhibition.

In some studies (58, 59) the sensitivity to plasma proteins inhibition of semi-synthetic surfactants (Kl-4 Surfactant or Surfaxin®, based on recombinant surfactant protein-B and Venticute® based on recombinant surfactant protein-C) was compared to other preparations which are on the market. Data from these studies support the hypothesis that the synthetic hydrophobic proteins can be used to design new surfactant preparations relatively resistant to inactivation.

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Received: 26 March 2003
Accepted in original form: 24 April 2003
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