
New Synthetic Surfactants: The Next Generation?

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Key Words

Respiratory distress syndrome · Preterm infant · Pulmonary surfactant · Surfactant proteins · Lucinactant · Lusupultide · SP-C analogs · ARDS · Clinical trials

Abstract

Surfactant preparations have been proven to improve clinical outcome of infants at risk for or having respiratory distress syndrome (RDS). In clinical trials, animal-derived surfactant preparations reduce the risk of pneumothorax and mortality when compared to non-protein-containing synthetic surfactant preparations. In part, this is thought to be due to the presence of surfactant proteins in animal-derived surfactant preparations. Four native surfactant proteins have been identified. The hydrophobic surfactant proteins B (SP-B) and C (SP-C) are tightly bound to phospholipids. These proteins have important roles in maintaining the surface tension-lowering properties of pulmonary surfactant. Surfactant protein A (SP-A) and D (SP-D) are extremely hydrophilic and are not retained in the preparation of any commercial animal-derived surfactant products. These proteins are thought to have a role in recycling surfactant and improving host defense. There is concern that animal-derived products may have some batch-to-batch variation regarding the levels of native pulmonary surfactant proteins. In addition, there is concern regarding the hypothetical risk of transmission of viral or unconventional

infectious agents from an animal source. New surfactant preparations, composed of synthetic phospholipids and essential hydrophobic surfactant protein analogs, have been developed. These surfactant protein analogs have been produced by peptide synthesis and recombinant technology to provide a new class of synthetic surfactants that may be a suitable alternative to animal-derived surfactants. Preliminary clinical studies have shown that treatment with these novel surfactant preparations can ameliorate RDS and improve clinical outcome. Clinicians will need to further understand any differences in clinical effects between available products.

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Introduction

Since the first use of surfactant in human neonates over two decades ago [1], numerous surfactant preparations have been developed and tested. These include animal-derived products and non-protein-containing synthetic products. Clinical trials of intratracheal administration of these exogenous surfactant preparations to very low birth weight or premature infants have shown in decreased mortality, reduced severity of respiratory distress syndrome (RDS), decreased likelihood of pneumothorax, and increased survival without chronic lung disease (CLD) [2]. Surfactants have gained universal acceptance and are credited with improvements in overall mortality, morbidity, and resource utilization [3, 4]. Evaluation of

new surfactant products using synthetic peptides or proteins has recently begun. The following article will briefly review the status of a variety of products that have added these proteins or peptides to surfactant preparations.

Surfactant Composition

Pulmonary surfactant in situ is largely uniform in all mammalian species [5]. It is synthesized in the type II alveolar epithelial cell and composed of several phospholipids, neutral lipids, and surfactant specific proteins. The largest proportion of phospholipid is phosphatidylcholine, of which dipalmitoylphosphatidylcholine (DPPC) is the major surface-active and most prevalent component [6]. The DPPC monolayer stabilizes the lungs by reducing the deflating force in the alveolus. However, the properties of pulmonary surfactant are not determined by the phospholipids alone. Surfactant proteins play an essential role in the biologic activity. Four native surfactant proteins have been identified. The hydrophobic surfactant proteins B (SP-B) and C (SP-C) are tightly bound to phospholipids. These proteins are found in all animal-derived surfactant products and are thought to have a role in the surface tension-lowering properties of pulmonary surfactant. Surfactant proteins A (SP-A) and D (SP-D) are hydrophilic and are not retained in the preparation of any commercial animal-derived surfactants. These proteins may play a role in surfactant metabolism and in the lung's host defense.

Animal Derived Surfactant Preparations

Advantages

In contrast to SP-A and SP-D, SP-B and SP-C are smaller, markedly hydrophobic proteins [7]. The hydrophobic nature of SP-B and SP-C protects these very important proteins from being lost in commercial, animal-derived, surfactant preparations. These two proteins are thought to be crucial in promoting the adsorption and spreading of monolayers of DPPC [8]. Their role in structural durability and functional organization of the phospholipids seems to be pivotal in the clinical superiority of animal-derived surfactants over non-protein-containing synthetic surfactants. This was demonstrated by clinical trials that compared a protein-free synthetic surfactant (colfosceril palmitate) to a protein-containing modified bovine minced lung surfactant extract (beractant). In-

fant receiving protein-containing beractant demonstrated a lower average FiO_2 , a lower mean airway pressure, a decreased incidence of air leak and respiratory disease compared to the group of infants receiving colfosceril palmitate [9, 10]. In another study there were similar results in the prophylactic treatment of infants at high risk of RDS [11]. Systematic review of clinical trials comparing animal-derived surfactant preparations to non-protein-containing synthetic surfactant preparations demonstrates that non-protein-containing synthetic surfactants are less effective than animal-derived products in terms of immediate ventilator support and the risk of pneumothorax and mortality. In addition, there is a marginal decrease in CLD among preterm newborns treated with animal-derived surfactant preparations [12].

Limitations

SP-B and SP-C are found in animal-derived surfactants, whether they are prepared from minced lung extracts (such as poractant alfa or beractant) or from lung lavage surfactant extracts (such as calf lung surfactant extract, calfactant, and SF-R11). However, the levels of SP-B and SP-C found in the commercial surfactant preparations vary from batch to batch and are reduced compared to levels found in native pulmonary surfactant [13, 14].

It has been postulated that the concentration of surfactant proteins in these preparations may sometimes be too low to support the optimal adsorption of phospholipids at the air-fluid interface. Synthetic surfactant proteins, or their functional analogs, might provide an opportunity to standardize the protein composition and thereby optimize surfactant function [15]. Synthetic protein-lipid surfactants produced by a molecular design approach would have a highly reproducible composition and could be more affordable.

Another potential limitation of animal-derived surfactants is the hypothetical risk of transmission of viral or unconventional infectious agents derived from an animal source. Although to date there has been no such documented complication, synthetic surfactants fortified with synthetic surfactant proteins would eliminate this potential risk. Furthermore, animal-derived surfactant proteins may be seen as foreign by the infant's developing immune system. Antibodies to these animal proteins may inactivate the exogenous surfactant as well as the native pulmonary surfactant [16]. In a near-term rabbit model, antibodies developed against animal-derived SP-B caused respiratory distress and acute inflammatory and exudative lung lesions, including hyaline membranes [17].

Protein-Containing Synthetic Surfactants

Surfactant preparations composed of synthetic phospholipids and essential hydrophobic surfactant protein analogs or mimics have recently been developed. These surfactant protein mimics have been produced by peptide synthesis and recombinant technology to provide a new class of synthetic surfactants that may be a suitable alternative to animal-derived surfactants. These surfactant preparations include peptides that, when added in an aqueous dispersion of phospholipids, function in a similar fashion as endogenous pulmonary surfactant proteins. The most promising efforts to date have been focused on the reproduction of the properties of SP-B and/or SP-C.

Surfactant Preparations Containing SP-B Mimics

Synthetic surfactant preparations that contain peptide fragments mimicking polypeptide segments of SP-B have been developed. The most extensively studied is lucinactant (Surfaxin[®], Discovery Laboratories, Inc., Warrington, Pa., USA). Lucinactant contains phospholipids and a peptide that is suggested to mimic a repeating pattern of hydrophobic and hydrophilic residues in the C-terminal part of SP-B. This peptide is called sinapultide (developmental name KL₄ peptide). Sinapultide consists of a stretch of four hydrophobic leucines (L) interspersed with cationic lysine (K) to create a 21-residue sequence KLLLLKLLLLKLLLLKLLLLK, believed to stabilize the phospholipid layer by interactions with the lipid heads and the acyl chains [18].

In vitro studies have demonstrated the ability of lucinactant to resist inhibition in a pulsating bubble surfactometer to a greater extent than animal-derived surfactant preparations [19–21]. In addition, the cationic peptide sinapultide induces surface phase separation of the lipid monolayer and enhances the ability to sustain high surface pressures [22].

In vivo studies have established the efficacy and safety of this synthetic peptide surfactant. Merritt et al. [23] successfully demonstrated homogenous pulmonary distribution. In a study comparing lucinactant to non-peptide-containing synthetic surfactant in preterm rhesus monkeys, Revak et al. [24] showed that lucinactant successfully expanded the pulmonary alveoli and promoted gas exchange.

Another synthetic peptide analog has been created utilizing an N-terminal segment of SP-B, SP-B_{1–25}, which induces monolayers when added to phospholipids in a fashion similar to full-length SP-B [25]. This peptide has been disulfide-linked into a homodimer, dSP-B_{1–25}. Pre-

term rabbits given dSP-B_{1–25} and synthetic phospholipids had consistently higher tidal volumes than those given either beractant or monomeric SP-B_{1–25} [26]. Although its biochemical characteristics look promising, dSP-B_{1–25} has not yet been tested in humans.

Surfactant Preparations Containing SP-C Analogs

Although SP-B seems to have the most important surface-active effect on phospholipids, there is no consensus regarding the relative importance of SP-B versus SP-C in terms of efficacy for clinical use. Phospholipids mixed with synthetic analogs of SP-C or with recombinant SP-C have been developed. Native SP-C is extremely hydrophobic and residues 9–34 form an α -helix. The α -helical structure is crucial to maintaining biophysical activity similar to native SP-C. Several SP-C analogs have been created of varying peptide length and using residue substitution to preserve the α -helical secondary structure and biophysical activity (see preceding article in this volume by Curstedt and Johansson for details).

Lusupultide is another novel synthetic surfactant preparation that uses recombinant SP-C (Venticute[®], Altana Pharma, Konstanz, Germany). Recombinant SP-C (rSP-C) is the 34-amino acid human SP-C sequence altered by the replacement of cysteine by phenylalanine in positions 4 and 5 and of methionine by isoleucine in position 32. In vitro, lusupultide was shown to effectively lower surface tension, more so than natural sheep surfactants. In addition, in vivo studies using two common preterm animal models of surfactant deficiency (lambs and rabbits) demonstrated similar PaCO₂, lung mechanics, and compliance when compared to animals treated with natural surfactants [27].

Surfactant Preparations Containing SP-A and SP-D Mimics

Efforts to simulate SP-B and SP-C have been the focus of most research due to their surface tension-lowering properties; however, the effect of SP-A and SP-D should not be overlooked. SP-D has immunomodulatory and host defense characteristics. It has been postulated that SP-D may be useful in reducing inflammation in the lung. SP-D-deficient mice have delayed removal of apoptotic cells such as alveolar macrophages, an important step in the resolution of inflammation. Recombinant SP-D has been shown to bind preferentially to these apoptotic cells and may have a role in reducing inflammation in future synthetic surfactant preparations [28]. Although SP-A is hydrophilic and not found in any commercial surfactant, one segment, residues 114–144, has been shown to be

highly hydrophobic. A 31-residue synthetic peptide analog (A114–A144) has been created to mimic this segment. This hydrophobic mimic enhances surface activity on a Langmuir/Wilhelmy surface balance and, when combined with synthetic phospholipids, enhances pulmonary compliance in preterm newborn rabbits [29].

Synthetic Surfactants in Acute Respiratory Distress Syndrome

Surfactant deficiency secondary to immaturity is the major emphasis for developing new synthetic surfactants; however, other conditions may be amenable to surfactant treatment. Inhibition or inactivation of pulmonary surfactant occurs in other pathologic states of newborns including meconium aspiration syndrome (MAS), sepsis, pneumonia, and perinatal asphyxia. These conditions may lead to acute respiratory distress syndrome (ARDS). In MAS, fatty acids, cholesterol, bile salts, bilirubin, and proteolytic enzymes in meconium may contribute to the alteration of surfactant function. Meta-analysis of the randomized controlled trials using animal-derived surfactants in treatment of MAS demonstrates a reduction of severity of respiratory illness and a decrease in the number of infants with progressive respiratory failure requiring support with ECMO [30]. The protein-containing synthetic surfactants may be effective in MAS as well. Adult rabbits and newborn rhesus monkeys with induced MAS treated with dilute lucinactant via repeated pulmonary lavage had clearing on chest radiographs and rapid improvement in pulmonary function [31]. A pilot study of the treatment of newborns suffering from MAS with dilute lucinactant showed the therapy to be safe and well tolerated. A trend towards lower oxygenation indexes and earlier extubation was noted [32]. The FDA has approved a phase III trial to study this further.

Similarly, infection may hamper surfactant function by the presence of serum proteins, blood products, and phospholipases in the airspaces. In particular, fibrinogen and oxidants released by the inflammatory process likely play a role in the inactivation of surfactant proteins. However, meta-analysis of trials of surfactant in the treatment of adult ARDS demonstrated no benefit despite evidence of surfactant dysfunction [33]. Despite this study, hope remains for a role of synthetic surfactants in the treatment of ARDS by virtue of increased resistance to inhibition. In a saline lung lavage piglet model of ARDS, lucinactant given as lavage was shown to improve pulmonary function and reduce protein leak [34]. Subsequently, lucinac-

tant was used to treat 12 adult patients with ARDS via lavage administration. The treatment was well tolerated and resulted in decreased oxygenation needs and lower ventilator requirements [35].

Other surfactants containing peptide fragments that mimic segments of SP-B have been tested in animal models of ARDS. dSP-B₁₋₂₅ surfactant has been evaluated in a saline lung lavage model of ARDS. Rats treated with this surfactant had better oxygenation and lung volumes compared to animals receiving beractant [26].

Synthetic surfactants based on SP-C analogs have been tested in treatment of non-RDS respiratory disease. Luspultide was shown to be at least as effective as bovine-derived surfactant preparations in a rat lavage model of ARDS [36].

Clinical Trials Using Protein-Containing Synthetic Surfactants

Most of the clinical experiences with protein-containing synthetic surfactant come from studies of neonates with RDS. A pilot trial using lucinactant for RDS in preterm infants demonstrated improved gas exchange, decreased requirement for assisted ventilation, and decreased mortality at rates proportional to those documented in previously conducted randomized controlled trials [37]. This study was limited by its small size and the lack of a randomized, masked control.

Two Phase III trials using lucinactant to prevent neonatal RDS have recently been completed. The 'SELECT' trial is a multicenter, international, randomized, controlled study comparing lucinactant to both colfosceril palmitate (Exosurf), a non-protein-containing surfactant, and to beractant (Survanta), a modified bovine-derived surfactant that contains SP-B and SP-C [38]. 1,294 intubated infants, 24–32 weeks gestation, with birth weights between 600 and 1,250 g, underwent computer-generated randomization in a masked manner to receive lucinactant, colfosceril palmitate, or beractant in a 2:2:1 ratio. Randomization was stratified based on birth weight. The drugs were delivered in covered syringes and a sham treatment of air was given in the case of dosing frequency discrepancies, since the interval for retreatment varies between the surfactant products.

The primary endpoints were occurrence of RDS at 24 h and occurrence of death related to RDS by 14 days. RDS was defined as the requirement for an $FiO_2 \geq 0.30$ at 24 h of age, associated with a chest radiograph with a reticulogranular pattern. RDS-associated death included

all deaths associated with pulmonary hemorrhage, respiratory failure, or air leaks. The sample size was calculated based on a prevention trial comparing colfosceril palmitate to calf lung lavage extract [11].

The groups of enrolled infants had similar clinical and demographic characteristics, with similar rates of chorioamnionitis, diabetes, pregnancy-induced hypertension, C-section, rupture of membranes, mean gestational age, and birth weight. However, infants who received beractant were less likely to have received antenatal steroids (74.3%) than lucinactant (79.2%) or colfosceril (78.5%).

On average, infants were treated before 30 min of age. In the intention-to-treat analysis, using the authors prespecified outcomes, 39.1% of the lucinactant group and 47.2% of the colfosceril palmitate group had RDS at 24 h. 4.7% of the lucinactant group and 9.4% of the colfosceril palmitate group died due to RDS-related causes (RR 0.50, 95% CI 0.32, 0.80). In the reference surfactant, beractant, group 33.3% developed RDS at 24 h and 10.5% had RDS-related mortality through 14 days (RR 0.45, 95% CI 0.27, 0.76).

Although RDS-related mortality is of interest, other more relevant clinical outcomes are also presented. All-cause mortality at 36 weeks' postmenstrual age (PMA) was not significantly different in either comparison: 21.1% for lucinactant vs. 23.8% for colfosceril palmitate and 26.4% for beractant. A trend towards a reduction in BPD or death at 36 weeks adjusted age was associated with lucinactant treatment when compared to colfosceril (RR 0.88, 95% CI 0.77, 1.01).

The 'STAR' trial is a multicenter, multinational, randomized, controlled study comparing prophylactic administration of lucinactant (Surfaxin) to poractant alfa (Curosurf), a protein-containing porcine-derived surfactant. 252 infants born between 24 and 28 weeks' gestation and weighing between 600 and 1,250 g were studied [39]. Randomization was accomplished using opaque sealed drug identification envelopes. Infants were stratified by birth weight (600–1,000 g, 1,001–1,250 g). Like the SELECT Trial, an independent dosing team was used to maintain appropriate masking of treatment assignment.

Poractant alfa has been tested and is commercially available in both 100 mg/kg and 200 mg/kg formulations. The 200 mg/kg version has been shown to significantly reduce mortality at 36 weeks' PMA compared to the 100 mg/kg version [40]. In an effort to give identical amounts of phospholipids to both poractant alfa and lucinactant groups, the investigators of the STAR trial chose to compare formulations containing 175 mg/kg. Although the tested preparation of poractant alfa is be-

tween two commercially available and effective preparations in phospholipid concentration, a more clinically relevant approach would be to follow the manufacturer's recommendation and test the best available clinical product.

The investigators structured the trial as a 'non-inferiority trial'. In order to model the data for non-inferiority, the investigators referenced historical data regarding the primary outcome (survival to 28 days without bronchopulmonary dysplasia (BPD)) in infants from a study comparing poractant alfa to placebo control [41]. In the investigator's construct, for lucinactant to be deemed 'non-inferior', the lower margin of the 95% CI of the treatment difference between lucinactant and poractant alfa would need to be at least 50% of the treatment difference observed in the historical trial of poractant alfa versus placebo.

The study was not reported based strictly on 'intention-to-treat'. Nine infants were randomized but did not receive surfactant in the prescribed time limit. These infants were not included in the analysis. However, there were similar numbers of excluded infants in both groups making it unlikely that this affected the analysis in a meaningful fashion.

The stated primary outcome was survival to 28 days without BPD and secondary outcomes were complications related to prematurity. The incidence of BPD at 28 days of life was 37.8% (95% CI 29.1%, 46.5%) for the lucinactant group compared to 33.1% (95% CI 24.8%, 41.3%) in the poractant alfa group. These confidence intervals meet the proposed definition of 'non inferiority'. However, clinicians will need a more clinically relevant measure to determine the relative efficacy of these preparations. The unadjusted relative risk and risk difference for the outcome BPD or death at 28 days demonstrates that further research will be needed to refine the estimate of the differences in efficacy of these two preparations (RR 0.99, 95% CI 0.77, 1.27).

The authors report other clinically relevant outcomes. The incidence of all-cause mortality at 36 weeks' PMA was 16% in the lucinactant infants and 18.5% in the poractant alfa infants (RR 0.92, 95% CI 0.53, 1.61). This is an encouraging trend. However, the estimate leaves room for clinically relevant differences in either direction; it is unfortunate that the trial was stopped prematurely due to unexpectedly slow recruitment.

Limited clinical data are available concerning the usage of protein containing synthetic surfactants in acute lung injury. In one recent phase III clinical study of adult ARDS patients, lusupultide demonstrated an improve-

ment in blood oxygenation during the initial 24 h of treatment, but no improvement in overall mortality [42]. However, a trend towards reduced mortality was noted in a subgroup of patients suffering from primary lung injury.

Conclusions

Currently effective surfactants are animal-derived and contain SP-B and SP-C in varying proportions, all of which are more scarce than found in endogenous pulmonary surfactant. A new generation of protein-containing surfactants has been developed. These products include lucinactant, a wholly synthetic surfactant which contains a peptide meant to mimic SP-B. The data from these

clinical trials demonstrate relative efficacy and safety in this newest generation of surfactants, the protein-containing synthetic surfactants, for prevention of RDS. Clinicians will need to further understand both the differences in clinical impact and issues regarding use of these new products. Although protein-containing synthetic surfactants may well become a suitable alternative with significant advantages, further studies will be needed to refine our estimate of the clinical impact of these preparations. Furthermore, supplementary use of surfactants for respiratory insults other than RDS is being explored; the usage of the synthetic surfactants holds great promise in this subset of infants. As the existing surfactants have greatly reduced the mortality associated with prematurity, perhaps the synthetic surfactants may reduce the incidence of BPD and chronic lung injury.

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