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New Generation Surfactants, Are They A Hope For Better Outcome ?

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Abstract:

Animal derived natural surfactants have been used in the treatment of neonatal respiratory distress syndrome for nearly 25 years with great success, but new, better and cheap surfactants are still needed. These new surfactants are mostly synthetic and some have been used clinically while some are still under review and have not been approved for use in infants. Recombinant SP-C, lucinactant, CHF5633, MİNİ-B and SP-C33 are some of the new generation synthetic surfactants which are discussed in this review.

Keywords: newborn, surfactant, respiratory distress syndrome

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Introduction

Respiratory distress syndrome (RDS) is a significant cause of morbidity and mortality in preterm infants. RDS is caused by a deficiency, dysfunction, or inactivation of pulmonary surfactant (PS). RDS's incidence increases with decreasing gestational age, the risk being 60% in less than 28 weeks and 30% between 28 and 34 weeks of gestation (1).

In addition to optimal respiratory support in the form of continuous positive airway pressure (CPAP) or mechanical ventilation and good supportive care, surfactant replacement therapy (SRT) forms the mainstay in the management of RDS (1). Introduction of SRT in the management of RDS is one of the most important advancements in the field of neonatology.

Pulmonary surfactant (PS) is synthesized and secreted by alveolar epithelial type II (AEII) cells (2) which is a complex compound formed by proteins and lipids. Surfactant participates in a range of physiological processes such as reducing the surface tension, keeping the balance of alveolar fluid, maintaining normal alveolar morphology and conducting host defense.

Human PS is a complex consisting of 86% phospholipids, 8% neutral lipids, and 6% SP. Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (50%) and phosphatidylglycerol (PG) (8%) are the most important phospholipids for the surface activity of PS (3) Of all the protein components in the mixture, the hydrophobic surfactant proteins B (SP-

B) and C (SP-C) have an essential function in the spreading, adsorption, and stability of surfactant lipids. The mixture has unique spreading properties, promotes lung expansion during inspiration, and prevents lung collapse during expiration (4). PS has four main surfactant proteins, SP-A, B, C, and D. Hydrophilic SP-A and D are removed in the manufacturing process; thus, current animal-derived PSs that are clinically used to treat neonatal RDS contain only hydrophobic SP-B and C (5). The quality of an exogenous surfactant is dependent on both its ability to increase lung compliance and to stabilize the alveoli at end-expiration.

Two broad categories of surfactants are available for exogenous therapy: surfactants derived from animal sources or 'natural' surfactants; and synthetic surfactants (6,7,8,9).

Most of the commercially used artificial PSs are derived from animal (cow or pig) lung tissue. These animal-derived PSs contain surfactant protein (SP) from animal lung extracts, a point that raises several worrisome questions. Such PSs contain animal antigenicity, which might have potential infectious burden. Additionally, the slaughter of a large number of cows or pigs is needed to produce commercial vials, leading to increased production costs.

The mammalian surfactant preparations are purified and extracted with organic solvents from either lung minces or lung lavages. Their phospholipid concentration is above 80% and all contain the low molecular hydrophobic proteins SP-B and SP-C. The porcine minced lung extract poractant (Curosurf®) undergoes an additional purification step that removes neutral lipid, whereas free fatty acids and DPPC are added to the bovine minced lung extract beractant (Survanta®). These preparations are expensive and have a limited supply and therefore there is a need for synthetic surfactant substitutes which can be produced in large quantities at low cost. The lipid mixture preparations obtained by extraction of natural surfactant are very complex and contain at least 50 different species in widely varying amounts. Compared to this, phospholipid mixtures in synthetic surfactant preparations are primitive. One difference in composition is that naturally derived surfactants contain the two hydrophobic proteins SP-B and SP-C while synthetic preparations contain analogues of either SP-B or SP-C. It was recently shown that both SP-B and SP-C (or SP-C33, an SP-C analogue) are necessary to establish alveolar stability at end-

expiration in a rabbit RDS model, as reflected by high lung gas volumes without application of positive end-expiratory pressure (10).

Development of synthetic surfactant has been an interest since the 1970's (11,12).

The results of first synthetic surfactant trials were largely negative; both had used nebulized dipalmitoylphosphatidylcholine (DPPC), and there were no discernible beneficial clinical effects (13,14).

During the 1980s there were numerous randomized controlled trials of many different natural and synthetic surfactants, demonstrating reductions in pulmonary air leaks and neonatal mortality (15).

Treatment with animal-derived surfactant preparation has been shown to result in better clinical response than synthetic surfactant during the acute phase of RDS, as evidenced by rapid weaning of inspired oxygen, lower mean airway pressure, and lower air leaks (16).

The greatest difficulty has been to synthesize the surfactant proteins SP-B and SP-C due to a metastable secondary structure (SP-C) and a complex tertiary structure (SP-B). SP-B and/or SP-C are important for optimal surfactant activity because of their ability to improve the reduction of surface tension mainly obtained by DPPC (17,18).

A major goal of the researchers is to progress towards the development of fully synthetic lung surfactants that have high surface and pulmonary activity as well as an ability to resist biophysical inhibition by endogenous substances present in the lungs during inflammatory injury. Treatment with such preparations, available in unlimited amounts in contrast to animal-derived surfactants, would also completely eliminate the already very low and hypothetical risks of transmission of viral or unconventional infectious agents (19,20,21).

The main characteristics and components of the most studied and used synthetic and animal-derived surfactants are found in Table 1.

The purpose of this review is to evaluate the structure and effects of synthetic pulmonary surfactants.

First-generation synthetic surfactants

Colfosceril and Pumactant, two synthetic surfactants devoid of SPs have been used clinically, namely colfosceril (Exosurf, Glaxo SmithKline, Brentford,

Table 1. Main characteristics of surfactants commonly used or studied in neonates (22).

Surfactant	Family	Main phospholipids	Proteins	Phospholipid concentration (mg ml ⁻¹)	Suggested dose (ml kg ⁻¹)	Phospholipid per dose (mg kg ⁻¹)
Colfosceril	Synthetic	DPPC	No	13.5	5	67.5
Pumactant	Synthetic	DPPC, PG	No	40	1.2	100
Beractant	Animal-derived (bovine)	DPPC, PG	SP-B/SP-C	25	4	100
Calfactant	Animal-derived (bovine)	DPPC, PG	SP-B/SP-C	35	3	105
Poractant	Animal-derived (porcine)	DPPC, PG	SP-B/SP-C	80	1.25/2.5	100/200
Lucinactant	Peptide-containing synthetic	DPPC, POPG	KL4 as SP-B	30	5.8	175

Abbreviations: DPPC, dipalmitoylphosphatidylcholine; PG, phosphatidylglycerol; POPG, palmitoyloleoylphosphatidylglycerol; SP-B, surfactant protein B; SP-C, surfactant protein C.

UK) and pumactant (artificial lung expanding compound, Britannia Pharmaceuticals Ltd, Redhill, Surrey, England).

Colfosceril: Exosurf®, which combines DPPC with a nonionic detergent (tyloxapol) and a spreading agent (hexadecanol), has been the most widely used synthetic surfactant.

Animal-derived surfactants that contain surfactant proteins (Survanta, Infasurf, and Curosurf) perform clinically better than Exosurf, primarily in outcomes related to acute management of respiratory distress syndrome (RDS; faster weaning and pneumothorax) but not in overall mortality or incidence of bronchopulmonary dysplasia (BPD) (23).

Exosurf® is not anymore used in most countries in Europe and North America, mainly because animal-derived surfactants have demonstrated decreased risks of death and pneumothorax, compared to protein-free synthetic surfactants (23).

Pumactant: The manufacturer's instructions recommend that pumactant should be given as soon as possible after birth, at 1 h, and again at 24 h. Babies born at 25–29 weeks gestation were randomised to receive either poractant alfa or pumactant as soon after birth as possible, and at 12 h with further doses at the discretion of the physician. The results showed an excess mortality in the pumactant treated group and the trial was stopped

early by the Data Monitoring Committee. Pumactant has now been temporarily withdrawn from use (24).

Newer Generation Surfactant Preparations

The rationale for the development of protein containing synthetic surfactants includes both practical and theoretical considerations. The addition of both surfactant protein B and C analogs to the phospholipid mixture will stabilize the alveoli, measured as lung gas volumes at end expiration, even if no positive end-expiratory pressure is applied. The effect on lung gas volumes seems to depend on the structure of the peptides as well as the phospholipid composition (25)

Synthetic surfactants would have highly reproducible composition with potentially less batch-to-batch surfactant protein (or mimic) variability (26). Furthermore, synthetic surfactants may lessen the risk of inflammation (27) and immunogenicity (28) associated with animal derived surfactants, as well as the theoretical risk of infection. New generation synthetic surfactants represent a promising alternative in the treatment of respiratory distress syndrome in preterm infants.

Classification of Synthetic Surfactants Based on Peptide Content is shown on Table 2 (5).

Table 2. Classification of Synthetic Surfactants Based on Peptide Content (5).

Based on simplified peptides	Based on surfactant protein-B analogs	Based on SP-C analogs
<ul style="list-style-type: none"> • WMAP10 • KL4-surfactant (Surfaxin) • Poly-N-substituted glycines (peptides) with α-chiral side chains 	<ul style="list-style-type: none"> • Peptides that cover the C-terminal partus • Peptides that cover the N-terminal partus • dSP-B1-25 • Mini-B 	<ul style="list-style-type: none"> • Synthetic SP-C analogs with native poly-Val sequence • Poly-Val \rightarrow poly-Leu-substituted SP-C analog [SP-C(Leu)] • SP-C (LKS) • SP-C33 • SP-C30

Recombinant SP-C (Venticute)

Lusupultide (Venticute, Altana Pharma) is a synthetic surfactant preparation that contains recombinant SP-C (rSP-C) and phospholipids. This has 2% rSP-C, DPPC/POPG in a ratio of 7:3 and 5% palmitic acid. It contains 50 mg phospholipids per ml and is a liquid suspension (21).

This recombinant surfactant protein C-based surfactant has been found to have favorable results in preclinical studies (29, 30) and two phase 1–2 clinical studies have shown a trend toward benefit (31).

In vitro, Lusupultide was shown to lower surface tension more than sheep surfactant extracts. In addition, in vivo studies of lusupultide utilizing two common preterm animal models of surfactant deficiency (lambs and rabbits) demonstrated similar improvement in ventilation, lung mechanics, and compliance when compared to animal derived surfactants (32).

In a recent study, Wemhöner and colleagues searched the effects of an animal-derived surfactant (Curosurf) and a synthetic surfactant (Venticute) on lipopolysaccharide (LPS)-induced inflammation were tested in human monocyte THP-1 cells. The effects were measured as changes in messenger RNA (mRNA) expression of the chemokine interleukin-8 (IL-8), proinflammatory TNF-alpha and the anti-inflammatory IL-10 cytokine. Both surfactant preparations inhibited the LPS-induced increase in TNF-alpha expression. A comparison of both preparations revealed a similar effect on IL-10 expression. However, IL-10 expression was higher after incubation with Venticute. Curosurf increased IL-8 expression at higher concentrations, but Venticute had no effect (33).

Also rSP-C surfactant has been studied in adults with ARDS (34) but not in neonatal RDS.

The tendency of native SP-C to aggregate into insoluble amyloid-like fibrils when separated from lipids, has limited its investigation and usage (35).

Lucinactant

Lucinactant was previously called KL4 surfactant, which contains 2.7% KL4, DPPC/POPG in a ratio of 3:1 and 13.5% palmitic acid. It also contains 30 mg phospholipids per ml and is suspended in buffer at pH 7.6 forming a gel until warmed to 44 °C (36,37). Lucinactant is the first clinically approved peptide-based pulmonary surfactant. Lucinactant (Surfaxin®), which contains synthesized KLLLL peptide mimicking human SP-B, was approved by the Food and Drug Administration in 2012 (36).

Surfaxin, its formulation with PS lipids, shows the promise of synthetic PS for replacing animal-derived PS in the treatment of respiratory distress syndromes and for treating acute lung injury. Efforts to characterize the molecular basis for KL4 function have revealed the peptide exhibits a helical structure which differentially partitions in response to both lipid saturation levels and pH. The penta-residue repeat of KL4 leads to adaptive peptide helicity, varying with partitioning depth, and suggests structural plasticity may represent an important mechanism for differential trafficking of lipids, particularly in intra-alveolar surfactant for the formation of stable DPPC monolayers at air-water interfaces (38).

KL4 has been described as an SP-B mimic but it seems to form a transmembrane α -helix making it more likely to function as an SP-C mimic (21).

Especially *in vitro* studies suggest it might be more resistant to inactivation by oxidation or serum proteins (39,40).

Similar to the native protein, the synthetic peptide induces molecular ordering of the phospholipid layer by contracting hydrophobic and electrostatic interactions with the phospholipids. This synthetic surfactant is more resistant to inhibition by meconium than beractant (Survanta®) or poractant (Curosurf®) (41).

This new generation surfactant is also used in clinical trials. A pilot study demonstrated that administration of lucinactant (Surfaxin®) to premature newborns with RDS is followed by a dramatic and long-lasting improvement in oxygenation similar to that observed with natural surfactants (42).

A recent meta-analysis of the two trials, comparing Lucinactant to animal derived surfactant extracts for the prevention of RDS (prophylactic strategy), showed no statistically significant difference in death, the primary outcome of interest (at 28 days: RR=0.79, 95% CI=0.61, 1.02; at 36 weeks PMA: RR=0.81, 95% CI=0.64, 1.03) (43).

Two recent trials evaluated and compared survival and pulmonary and neurodevelopmental outcomes through 1 year corrected age of preterm infants who received lucinactant and other surfactants in the SELECT (Safety and Effectiveness of Lucinactant Versus Exosurf in a Clinical Trial) and STAR (Surfaxin Therapy Against Respiratory Distress Syndrome) trials individually and, secondarily, from analysis using combined data from these 2 trials.

Findings from this 1-year follow-up of both lucinactant trials indicate that this new peptide-based synthetic surfactant is at least as good, if not superior, to animal-derived surfactants for prevention of respiratory distress syndrome and may be a viable alternative to animal-derived products (44). Also the meta-analysis demonstrated a statistically significant decrease in the rate of necrotising enterocolitis in infants who received Lucinactant, compared to those who received animal-derived surfactants (RR=0.48 95% CI=0.30, 0.74). There was no statistically significant reduction in other major outcomes such as BPD at 36 weeks PMA, death or BPD at 36 weeks PMA, duration of ventilation and supplemental oxygen, and air leaks. One-year follow-up data have been recently reported no statistically significant differences in the rates of blindness, deafness, and mortality in infants who received Lucinactant

compared to those who received an animal derived surfactant extract (44).

Recent phase 3 clinical trials with Surfaxin show promising results with similar efficacy as animal derived surfactants and yet avoiding the disadvantage associated with animal products (45).

Clinical and synthetic surfactant therapy reduces alveolar-capillary protein leakage in surfactant-deficient rabbits. Surfactant preparations with both SP-B and SP-C (analogs) were more efficient than preparations with SP-B or SP-C alone (46).

A lyophilized form of Surfaxin as well as an aerosolized version, known as Aerosurf, are currently in clinical trials for the treatment of RDS (38).

The present results are encouraging for the development of a fully synthetic surfactant that can be used in replacement therapy.

CHF5633

CHF5633 is the new totally synthetic surfactant synthesized by Chiesi Farmaceutici S.p.A (Parma, Italy) for the treatment of preterm newborn infants with RDS. CHF5633 contains DPPC and POPG 1:1 ratio (98.3%), surfactant protein (SP)-C analogue (1.5%), and SP-B analogue (0.2%). High content of PG in CHF5633 might be beneficial for prevention of chronic lung injury. CHF5633 is a white uniform suspension, stable at room temperature, has low viscosity, and does not require any heating before use. Commercially available surfactants and CHF5633 do not contain the collectin family members SP-A and SP-D, which play important roles in host defense (47).

CHF5633 was evaluated using immature newborn lamb model and compared with animal lung tissue-based surfactant Survanta. This study demonstrated for the first time that new synthetic surfactant CHF5633 dramatically improved lung function of preterm newborn lamb lung, similarly as animal lung tissue-based surfactant. Furthermore, there were slight but significant beneficial effects of CHF5633 over Survanta on improvement of tidal volume and compliance immediately after treatment (48).

Its biophysical effectiveness has been demonstrated *in vitro* and *in vivo*. CHF5633 also has possible immunomodulatory abilities.

In a recent trial highly purified adult CD14+ cells, either native or simultaneously stimulated with LPS, were exposed to CHF5633, its components, or

poractant alfa (Curosurf®). Subsequent expression of TNF- α , IL-1 β , IL-8 and IL-10 mRNA was quantified by real-time quantitative PCR, corresponding intracellular cytokine synthesis was analyzed by flow cytometry. CHF5633 did not exert unintended pro-inflammatory effects in both settings. On the contrary, CHF5633 significantly suppressed TNF- α mRNA expression in LPS-stimulated adult monocytes, indicating potential anti-inflammatory effects (49).

In Fehrholz and colleagues' study, purified human CD4+ T cells were activated using anti CD3/CD28 antibodies and exposed to CHF5633, its components, or to the well-known animal-derived surfactant Poractant alfa (Curosurf®) and the immunomodulatory capacity of CHF5633 on CD4+ lymphocytes was evaluated. CHF5633 did not show any cytotoxicity on CD4+ cells. Moreover, in vitro data indicate that CHF5633 does not exert unintended pro-inflammatory effects on non-activated and activated CD4+ T cells (50).

Another recent study confirms that CHF5633 does not exert unintended pro-apoptotic and pro-inflammatory effects in human neonatal monocytes. CHF5633 rather suppressed LPS-induced TNF- α and IL-1 β cytokine responses (51).

This new generation of surfactant made of these analogues combined to phospholipids has been evaluated through randomized controlled trials with promising results.

MINI-B

Dimeric SP-B1–25, a homodimer of a 25–amino acid residue peptide based on the N-terminal of human SP-B, produces a range of physical changes in lung surfactant monolayers similar to those observed for native SP-B, including an enhanced coexistence of buckled and flat monolayers (52).

Synthetic surfactant preparations containing dimeric SP-B1–25 improve lung function in animal models of neonatal RDS and acute RDS (ARDS) (53) but not yet studied in humans.

A recent animal study examines the surface activity, resistance to biophysical inhibition, and pulmonary efficacy of a synthetic lung surfactant containing glycerophospholipids combined with Super Mini-B (S-MB) DATK, a novel and stable molecular mimic of lung surfactant protein (SP)-B. In addition to directly documenting the beneficial inhibition-resistance of S-MB DATK surfactant, another

important aspect of this study was to directly define the in vivo effectiveness of this synthetic preparation when instilled intratracheally into animals with lung injury and surfactant deficiency relevant for the neonatal respiratory distress syndrome (NRDS) and ALI/ARDS (54). Synthetic surfactants containing S-MB DATK (or related peptides) combined with lipids appear to have significant future potential for treating clinical states of surfactant deficiency or dysfunction, such as neonatal and acute respiratory distress syndromes.

SP-C33:

This synthetic surfactant contains 2% SP-C33, DPPC/POPG in a 7:3 ratio but no palmitic acid. It is a liquid suspension and can be concentrated to 80 mg phospholipids per ml (21). SP-C33 is currently being studied in Stockholm but no clinical trials have yet been reported.

Conclusion

Surfactant therapy has been a major contribution to care of the preterm newborn during the past 25 years. Introduction of SRT in the management of RDS is one of the most important advancements in the field of neonatology.

Increased understanding of the surfactant proteins will hopefully lead to development of effective synthetic surfactants which can be produced in large quantities for treatment of a wide range of respiratory disorders

Recent intensive basic and clinical research has led to the development of peptides that mimic the function of the hydrophobic proteins SP-B and SP-C. A new generation of surfactants made of hydrophobic proteins SP-B and SP-C analogues combined to phospholipids has been evaluated through randomized controlled trials with promising results. It is likely that proteins containing synthetic surfactants with highly reproducible composition, produced in large amounts at a low cost, and not dependent on an animal source.

It seems that synthetic surfactants containing two peptides and a more complex phospholipid composition will be able to replace natural surfactants within the near future. Further well designed, independent studies of adequate size and power are needed to confirm newer generation synthetic surfactants' safety and efficacy.

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