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# New Synthetic Surfactants – Basic Science

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## Key Words

Pulmonary surfactant · Synthetic surfactant ·  
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Respiratory distress syndrome · Lung disease

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## Abstract

The hydrophobic surfactant proteins, SP-B and SP-C, promote adsorption of surface-active lipids to the air-liquid interface of the alveoli and are essential for alveolar stability and gas exchange. Synthetic surfactant preparations must contain at least one of these hydrophobic proteins, or analogs thereof, to have optimal effects when administered into the airways of patients with lung diseases. However, development of clinically active artificial surfactants has turned out to be more complicated than initially anticipated since the native hydrophobic proteins are structurally complex or unstable in pure form. The proteins have been replaced by different analogs which have the right conformation without forming oligomers. 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. Furthermore, the lipid composition seems to be important, as well as a high lipid concentration in the suspension. For successful treatment of many respiratory diseases, it is also desirable that the synthetic surfactant resists inactivation by plasma components leaking into the alveoli.

## Introduction

Neonatal respiratory distress syndrome (RDS), caused by surfactant deficiency, is still a major cause of morbidity and mortality in preterm babies. Pulmonary surfactant is a complex of surface-active lipids, especially dipalmitoylphosphatidylcholine (DPPC), and at least four specific proteins, surfactant proteins A, B, C and D (SP-A, -B, -C and -D). A major function of surfactant is to reduce surface tension, thus preventing the alveoli from collapsing at end-expiration [1]. The two hydrophobic proteins SP-B and -C are critical for the adsorption and spreading of the surfactant film at the air-liquid interface. Although accomplished in cooperation with surfactant proteins, it is the lipids, especially DPPC, that are ultimately responsible for surface tension reduction to low values during expiration. The main function of the hydrophilic proteins SP-A and -D is to participate in pulmonary host defense [2, 3].

In preterm babies with surfactant deficiency, repeated collapse and expansion of the lungs can lead to mechanical disruption of airway epithelium and impairment of lung mechanics and gas exchange, which are found in RDS. Impaired surfactant activity is also a central feature in the pathophysiology of many types of acute lung injury.

Today, airway instillation of surfactant is in general use for treatment of RDS in preterm babies. The most satisfactory results have been obtained with preparations purified from animal lungs, in some cases modified by

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addition of specific lipids for improving functional properties. Common components in these preparations are phospholipids, mainly DPPC, and the hydrophobic SP-B and -C. Both proteins accelerate film formation at the air-liquid interface, probably by different mechanisms. The fundamental roles of both endogenous SP-B and -C in pulmonary surfactant have been verified by experimental and clinical observations. Gene targeting experiments have revealed that both SP-B and -C knockout mice are unable to establish normal breathing [4, 5]. Infants with SP-B deficiency develop progressive, lethal respiratory failure in the neonatal period, while infants with SP-C deficiency will develop progressive lung fibrosis [6].

Surfactant preparations derived from animal lungs are expensive with a limited supply and they pose a potential danger of propagating infectious material. Thus, there is a need for synthetic surfactant substitutes, which can be produced in large quantities at a reasonable cost. In acute lung injury, which can arise in association with meconium aspiration, sepsis, trauma, diffuse pneumonia and circulatory shock, surfactant is not deficient but plasma proteins leaking into the airspaces inhibit its activity. For successful treatment of these lung diseases, it is desirable that the instilled surfactant resists inactivation by different components.

### First Synthetic Surfactants

Surfactant replacement therapy in premature infants with RDS was first reported in the 1960s. The first synthetic surfactant preparations tested for clinical use were made from lipids only, simply because the surfactant proteins were not yet discovered. Aerosolized DPPC was used as a surfactant substitute without impressive effects. This may have been due to the fact that DPPC was used without a spreading agent, and therefore would not have been surface active at body temperature. During this time treatment of RDS with continuous positive airway pressure and artificial ventilation with positive end-expiratory pressure (PEEP) were evaluated.

Attempts have been made to produce synthetic surfactants with DPPC and spreading agents such as high-density lipoprotein [7], phosphatidylglycerol (ALEC) [8] or hexadecanol plus tyloxapol (Exosurf®) [9]. These surfactant preparations devoid of surfactant proteins have limited acute short-term therapeutic effects in infants with established RDS, but by entering the recycling machinery of type II cells they might slowly improve lung function and survival, especially when given prophylactically or

during the early course of the disease. ALEC, which was licensed in the UK, was withdrawn from the market following a UK trial comparing this surfactant with the natural surfactant Curosurf®, since a higher mortality in the infants treated with the synthetic surfactant was observed [10].

### Hydrophobic Surfactant Proteins

The biophysical and physiological activity of surface-active lipids can be greatly enhanced by addition of 1–2% of the hydrophobic SP-B and -C [11]. These proteins accelerate film formation at an air-liquid interface, probably by different mechanisms. When present in the surface film each of these proteins may recruit additional lipids from the hypophase and promote tight packing of the lipids at the air-liquid interface. SP-B and -C differ significantly from each other in their structural properties and in the way they interact with lipid membranes [1]. SP-B is a 17.4-kDa homodimer where each polypeptide chain may interact with a lipid bilayer. Thus, SP-B might be able to cross-link two adjacent lipid bilayers. SP-C is a 4.2-kDa lipopeptide with an N-terminal part containing two palmitoyl chains thioester linked to Cys5 and Cys6 and a middle/C-terminal hydrophobic  $\alpha$ -helix composed mainly of valine residues [12]. The  $\alpha$ -helix of SP-C is perfectly adapted to span a bilayer of fluid DPPC while the helix adopts a tilt of about 70° in a DPPC monolayer [13–15].

Development of clinically active synthetic surfactants has turned out to be more complicated than initially anticipated. SP-B is too big and structurally complex to be synthesized by organic chemical methods and expression of functionally active recombinant SP-B has not been reported. SP-C is extremely hydrophobic and, especially in pure form, structurally unstable [16].

A new generation of synthetic surfactants based on synthetic phospholipids and analogs of the hydrophobic surfactant proteins is currently under development. In contrast to natural surfactant preparations these synthetic surfactants are physiologically active under experimental *in vivo* conditions only when the animal is ventilated with PEEP [17], indicating that natural surfactant preparations are more effective in stabilizing the airways. This difference in activity may be masked in clinical practice since ventilation with PEEP is part of the routine management of patients with severe respiratory failure.

## Synthetic Surfactants Based on Simplified Peptides

McLean et al. [18] designed a series of helical amphipathic decapeptides, starting from the sequences of the lipid-binding regions of the plasma apolipoproteins. One of these peptides, WMAP10, in DPPC reportedly restored static pulmonary compliance and arterial-to-alveolar oxygen ratio in lung-lavaged animals almost completely. In vitro this preparation had very slow spreading kinetics at an air-liquid interface and a high maximum surface tension during pulsation in the bubble surfactometer. No further studies have appeared regarding this surfactant preparation.

KL<sub>4</sub> is a 21-residue peptide which was designed by Cochrane and Revak [19] to mimic the structure of SP-B. However, the peptide seems to form a transmembrane  $\alpha$ -helix in a phospholipid bilayer [20], thus indicating that it resembles SP-C rather than SP-B. In contrast to these results infrared reflection-absorption spectroscopy of KL<sub>4</sub>-containing phospholipid monolayer revealed an antiparallel  $\beta$ -sheet structure [21]. Thus, further studies are needed to find out if KL<sub>4</sub> in some aspects may be regarded as an SP-B or -C analog. Addition of KL<sub>4</sub> to Survanta<sup>®</sup> did not improve oxygenation in lung-lavaged adult rats as efficiently as did addition of synthetic SP-B<sub>1-78</sub> monomer and synthetic SP-C analog, or the SP-B<sub>1-78</sub> alone [22].

KL<sub>4</sub>-surfactant (Surfaxin) is a mixture of DPPC, palmitoyloleoylphosphatidylglycerol, palmitic acid and KL<sub>4</sub> [23]. Surfaxin has a gel structure that requires heating at 44°C for 15 min and subsequent shaking before tracheal instillation [24]. The surfactant increases dynamic lung compliance and oxygenation when tested in premature monkeys [25]. In a noncontrolled clinical trial KL<sub>4</sub> surfactant at a dose of 200 mg/kg was given to newborn babies with RDS resulting in an improvement of arterial-to-alveolar oxygen tension ratio [23].

Non-natural bioinspired structures that capture both the amino acid sequence patterning and the 3-dimensional folds of natural surfactant proteins have been proposed for replacement of the hydrophobic SP-B and -C [26, 27]. One type of these polymers is poly-N-substituted glycines (peptoids) with  $\alpha$ -chiral side chains which adopt a stable, helical secondary structure in organic and aqueous solutions and are nearly resistant to enzymatic degradation. These peptoids have been created to mimic both the SP-B fragment SP-B<sub>1-25</sub> [26] and the SP-C analog SP-C(Leu) [27, 28]. About 10% (w/w) of these non-natural molecules added to the lipid mixture DPPC:palmitoyloleoylphos-

phatidylglycerol:palmitic acid (68:22:9, by weight) improve surface pressure-area isotherms, surfactant film morphology and dynamic adsorption and decrease minimum and maximum surface tension during cycling in the pulsating bubble surfactometer. However, no data on the in vivo performance with these artificial surfactants have been published.

## Synthetic Surfactants Based on SP-B Analogs

Several peptides covering different parts of SP-B have been synthesized and their biophysical and physiological activities in different lipid mixtures have been evaluated in vitro and in vivo [29]. Peptides that cover the C-terminal part and have a length of at least 17 amino acids accelerate surfactant spreading and improve static lung compliance in premature newborn rabbits. Furthermore, N-terminal peptides, especially residues 1–25, seem to mimic most of the in vitro activities of native SP-B [30]. Dimeric N-terminal protein B, dSP-B<sub>1-25</sub>, enhances surface activity compared to monomeric SP-B<sub>1-25</sub> [31] and both in vivo and in vitro studies indicate that increasing the dSP-B<sub>1-25</sub> concentration from 1 to 2% in a lipid mixture improves surface activity, oxygenation and lung volume [32]. Addition of a synthetic palmitoylated SP-C analog optimizes these surfactants but is not absolutely required [32].

## Synthetic Surfactants Based on SP-C Analogs

SP-C contains only 35 amino acid residues which makes it suitable for solid phase peptide synthesis [12]. However, the molecule is extremely hydrophobic and, especially in pure form, structurally unstable [33]. These features have complicated the development of synthetic SP-C and resulted in the development of SP-C analogs which fold effectively into an  $\alpha$ -helical conformation [28, 34].

Full-length non-palmitoylated human SP-C analogs and various truncated forms in lipid mixtures have been evaluated in vitro and in vivo. The sequence of residues 5–31 or 6–32, which includes the hydrophobic helical structure in native SP-C, is necessary for biophysical activity [35]. Previous studies have shown that formation of a transmembrane  $\alpha$ -helix is more important than retaining the exact amino acid sequence [36]. Since the poly-valyl sequence does not favor helix conformation, analogs with substitutions in this region have been de-

signed. Replacement of the poly-valyl part of SP-C with a poly-leucyl stretch produces an analog (SP-C(Leu)) which forms an  $\alpha$ -helix [28, 37]. Airway instillation of this peptide combined with DPPC:phosphatidylglycerol:palmitic acid (68:22:9, by weight) increases dynamic lung compliance in premature newborn rabbits by about 30% [28]. Similar results are obtained with a synthetic SP-C analog composed of a poly-leucine stretch linked to the heptapeptide segment covering SP-C positions 6–12 [37, 38].

Several analogs based on the SP-C structure have been described. Synthetic SP-C analogs with the native poly-Val sequence are capable of forming effective surfactants [35]. Synthetic surfactant based on recombinant SP-C with the same amino acid sequence as human SP-C but lacking the palmitoyl chains is highly effective at restoring lung function in surfactant-deficient animals. In more recent studies, the structure has been simplified and Cys5 and Cys6 replaced by Phe, and Met32 by Ile. Surfactant based on this recombinant SP-C, improves lung function in premature newborn rabbits and lambs [17, 39] as well as in animal models of acute lung injury [40, 41]. However, treatment with recombinant SP-C surfactant in patients with acute RDS did not improve survival but improved gas exchange during the 24-hour treatment period [42]. In our experience, synthetic SP-C containing a poly-Val sequence exhibits low surface activity *in vitro*, mainly because this peptide does not fold into the helical conformation shown by SP-C isolated from lung tissue but has a strong tendency to form aggregates with the peptide in a  $\beta$ -sheet conformation [33, 36]. In order to overcome the difficulties a poly-Val $\rightarrow$ poly-Leu-substituted SP-C analog (SP-C(Leu)) was synthesized and found to be virtually identical to native SP-C in terms of secondary structure and *in vitro* surface-spreading properties [28]. SP-C(Leu)/lipid mixtures, however, only show a modest effect on dynamic lung compliance in premature rabbit fetuses. This was correlated with the fact that SP-C(Leu) oligomerizes into dimers, tetramers, hexamers and probably also higher-order oligomers, which makes it difficult to suspend SP-C(Leu)/lipid mixtures at concentrations higher than 20 mg/ml [28]. Moreover, a poly-Leu SP-C analog was synthesized by Takei et al. [37] and found to be active *in vivo*. The problem with peptide oligomerization was circumvented by synthesizing a modified peptide, SP-C(LKS), containing three lysines incorporated in the SP-C(Leu) sequence in such a way that they cover the helix circumference and reduce peptide-peptide interactions [43]. This peptide, SP-C(LKS), combined with lipids exhibits rapid adsorption and spreading [43], and can

be suspended at 80 mg/ml. However, instillation of this preparation does not significantly improve lung compliance of premature rabbit fetuses [unpubl. data]. The reason for this is not known but it is possible that introduction of positively charged Lys residues in the middle and C-terminal regions interferes with the strong SP-C dipole moment (from the intrinsic helix dipole and from the positively charged N-terminal residues) and/or influences the peptide/membrane interactions. The problems with peptide oligomerization and interference with the dipole moments were circumvented by synthesizing an SP-C analog (SP-C33) with one positive charge in the N-terminal part of the  $\alpha$ -helical chain.

SP-C33/lipid mixtures can be suspended and administered at a concentration of 80 mg/ml. The synthetic surfactant preparation increases lung compliance in preterm rabbit fetuses to levels similar to those obtained with commercially available modified natural surfactant preparations currently used for treatment of clinical RDS [34]. Administration of surfactants at high concentration is likely to be important for optimal treatment effects, as evidenced by the slow spreading observed at low phospholipid concentrations of natural surfactant preparations [44]. The surface activity also seems to depend on the lipid composition [34]. Furthermore, the airways seem to be stabilized less effectively with SP-C33 than by natural surfactant, and immature animals ventilated after treatment with the synthetic surfactant need PEEP for an optimal physiological response.

Histological examination shows that animals treated with SP-C33/lipid mixtures have no or only mild airway epithelial damage, comparable to the effects of treatment with modified natural surfactants. However, end-expiratory lung gas volumes are significantly larger in animals treated with Curosurf than in those receiving SP-C33/lipid mixtures and significantly larger in animals treated with PEEP than in those ventilated without PEEP [34]. The combination of relatively large tidal volumes and low end-expiratory lung gas volumes in animals receiving SP-C33/lipid mixtures indicates alveolar instability, which at least under the present experimental conditions can be compensated for by application of PEEP. Further studies are required to find out whether treatment effects on lung gas volumes can be improved by adding SP-B (or some analog of SP-B) to the synthetic surfactant, or by modifying the lipid composition.

## Resistance to Surfactant Inactivation

Endogenous and exogenous pulmonary surfactant may be inhibited by, for example, meconium or albumin. By addition of different components such as dextran [45, 46], polyethyleneglycol [46], hyaluronan [47] or polymyxin [48] to surfactant preparations, the inhibition can be counteracted as shown by both in vitro and in vivo experiments. Furthermore, recent observations in our laboratory have shown that the function of SP-B can be mimicked in vitro by polymyxin B, a structurally different acylated peptide, which cross-links phospholipid vesicles by ionic interactions. Addition of 2% (w/w) of polymyxin B to the surfactant preparation increased the resistance of pulmonary surfactants to inactivation and improved their surface properties [48]. These data indicate that one of the functions of SP-B is to cross-link lipid vesicles and that this effect can be mimicked by a rather simple peptide.

## Conclusions

The hydrophobic proteins SP-B and -C, together with surface-active phospholipids, are essential constituents of exogenous surfactant. However, SP-B is too big and structurally complex to be synthesized and SP-C is structurally unstable in pure form. Intensive research has pro-

vided increased understanding of molecular mechanisms involved in the formation and preservation of the surfactant film at the alveolar lining. These efforts have promoted the development of stable and rather simple peptides which may mimic the functions of SP-B and -C. New synthetic surfactant preparations based on these peptides and synthetic lipids will probably be available on the market in the near future and eventually replace natural surfactant preparations. Furthermore, indications for surfactant therapy will be widened. Besides treatment of newborn babies with RDS, surfactant therapy might be effective in newborns with inactivated surfactant in conditions such as meconium aspiration syndrome or neonatal group B streptococcal pneumonia. The synthetic surfactant may also be used as a carrier for antibiotics, specific antibodies or other drugs, for treatment of various lung diseases not primarily related to the surfactant system. To widen the indications it is important that the new synthetic surfactant preparations are well defined and can be produced in large amounts at a reasonable cost.

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