Occasional review

Surfactant replacement therapy

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In 1959 Avery and Mead discovered that the lungs of infants who died of hyaline membrane disease (HMD) were deficient in surfactant.[1] Surfactant reduces surface tension and stabilises alveoli at low lung volumes and may act as an "anti-stick" or lubricant allowing the alveolar walls to remain open.[2–4] HMD is characterised by progressive atelectasis and respiratory failure in premature infants and is a major cause of morbidity and mortality in the neonatal period.[5,6] Numerous studies of the development of the pulmonary surfactant system have led to the clinical use of phospholipid composition of human amniotic fluid to predict lung maturity and to the use of antenatal hormones to accelerate lung maturation. Recently, this research has culminated in successful attempts to treat HMD in premature infants with surfactant replacement therapy (SRT).

We will begin this article with a brief review of the pulmonary surfactant system and the various types of surfactants used in replacement therapy. This will be followed by a review of the animal and clinical studies investigating SRT in various disease conditions.

Biology of surfactant

As shown in table 1, surfactant contains 85–90% lipids (of which 85% are phospholipids and 5% are neutral lipids) and 10–15% protein.[2,7,8] The major phospholipid in surfactant is phosphatidylcholine of which approximately 70–75% is present as dipalmitoyl phosphatidylcholine (DPPC), the major surface active component in surfactant. The second most abundant phospholipid is phosphatidylglycerol (PG) which comprises 7–10% of total surfactant. These lipids are important in the formation of the monolayer on the alveolar-air interface, and PG is important in the spreadability over a large surface area.

Table 1 Composition of mature surfactant

	Percentage of total weight
Lipid	85–90
Protein	10–15
	Percentage of lipids
Phospholipids	85–90
Neutral lipids	5
Glycolipids	5–10
	Percentage of total phospholipids
Phosphatidylcholine	70–80
Dipalmitoyl phosphatidylcholine	45–50
Phosphatidylglycerol	7–10
Phosphatidylethanolamine	3–5

There are four specific surfactant proteins which comprise 2–5% of the weight of surfactant.[9,10] The most studied surfactant protein is SP-A which is expressed in alveolar type II epithelial cells and Clara cells in the lung.[11–13] The gene for this protein is found on chromosome 10 and contains four exons that encode the primary translation product of 26–28 kD.[9,10] Post translational glycosylation, hydroxylation of proline residues, and sialylation results in a glycoprotein doublet with apparent molecular weights of 28–30 kD and 34–36 kD.[9–11,14–18] The carboxy terminal globular glycosylated region contains two intramolecular disulphide bonds.[9,10] The N-terminal collagen-like regions from three monomers form a triple α-helix that is important for the assembly of the protein.[19,20] The fully assembled molecule contains six of these oligomers with an apparent molecular weight of approximately 650 kD.[21,22] SP-A appears to be involved in the regulation of surfactant turnover,[23–31] formation of tubular myelin,[32] and the immune regulation within the lung.[33,34]

There are two hydrophobic surfactant specific proteins, SP-B and SP-C.[9–11,35–38] The gene for SP-B resides on chromosome 2 and contains 10 exons.[9,10] The primary translation product is a 42 kD protein which is cleaved to an active peptide of approximately 7–8 kD under reducing conditions.[39] The function of SP-B is not well understood, but it may be involved in the formation of tubular myelin.[32] Recent studies have shown that SP-B is involved in the surface activity of surfactant and that this protein may increase the intermolecular and intramolecular order of the phospholipid bilayer, supporting the concept that SP-B resists surface tension by increasing the lateral stability of the phospholipid layer.[40] A recent study confirmed that the 25 amino-terminal peptides of SP-B stabilised the phospholipid layer by increasing the collapse pressure of surfactant phospholipids which may prevent squeezing out of the phospholipids from the monolayers at the alveolar-air interface.[41] A specific charge interaction between the cationic peptide and an anionic lipid such as PG may be responsible for this stabilisation.

The gene for SP-C resides on chromosome 8 and contains six exons resulting in a primary translation product of 21 kD.[9,10,42,43] This protein is cleaved into an active peptide of 4–5 kD.[43] SP-C may be involved in the spreadability and surface activity of surfactant, but its

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Table 2 Types of surfactant used in clinical studies

Classification	*Source*	*Surfactant protein content*
Natural surfactant	Human amniotic fluid	SP-A, SP-B, SP-C, SP-D
Modified natural surfactant	Bovine lung extract (Survanta, Surfactant TA)	SP-B, SP-C
	Bovine lung lavage extract (Infasurf)	
	Porcine lung extract (Curosurf)	
Synthetic surfactant	DPPC, PG, tyloxapol, hexadecanol (Exosurf)	None
	DPPC:PG, artificial lung expanding compound (ALEC)	

DPPC = dipalmitoyl phosphatidylcholine; PG = phosphatidylglycerol.

exact function remains unknown. The most recently discovered surfactant specific protein, SP-D, is a glycoprotein with an apparent molecular weight of 43 kD and contains an N-terminal collagenous domain and a carboxy terminal glycosylated domain similar to SP-A.[44-47] The function of SP-D is unknown at this time.

Pulmonary surfactant is synthesised in the alveolar type II epithelial cell and stored in the lamellar body.[48-50] Secretion of surfactant into the airway occurs via exocytosis of the lamellar body by both constitutive and regulated mechanisms.[2 48 51] Numerous agents have been shown to stimulate the secretion of surfactant including β adrenergic agonists, activators of protein kinase C, leukotrienes, and purinergic agonists.[51-55] The major route of clearance of pulmonary surfactant within the lung is through reuptake by type II cells with minor contributions occurring through absorption into lymphatics and clearance by alveolar macrophages.[56 57] After being taken up by the type II cell, the phospholipids are either recycled for resecretion or degraded and reutilised in the synthesis of new phospholipids.[58-62] These processes have recently been shown to be developmentally regulated in fetal lung.[26]

Preparations used in surfactant replacement therapy

There are three basic types of surfactant (natural, modified natural, and synthetic) that have been considered for SRT (table 2).[63 64] Natural surfactant from sheep and rabbits has been used in studies of the effects of SRT in preterm animals suffering from HMD. Several clinical trials in humans have used human natural surfactant which has been obtained from amniotic fluid from full term pregnancies. As would be expected, these natural surfactants contain all the phospholipids and neutral lipids as well as the surfactant proteins (SP-A, SP-B, SP-C, SP-D). A second type of surfactant used in SRT is the modified natural surfactant obtained from either bovine or porcine lung. These surfactants are obtained by organic solvent extraction of lung lavage or minced lung from bovine and porcine sources. The bovine preparations have been referred to as calf lung surfactant extract (CLSE or Infasurf), Surfactant TA, and the preparation which was approved by the FDA in 1991, Survanta. A porcine surfactant (Curosurf) has been used in Europe. In addition to containing the phospholipids of surfactant, these modified natural surfactants also contain the hydrophobic surfactant proteins SP-B and SP-C, but they do not contain the hydrophilic proteins, SP-A and SP-D. Synthetic surfactants have been designed and one preparation contains surface active phospholipids as well as agents which improve spreadability and stability of the preparation. The first synthetic surfactant preparation that was approved for use by the FDA in 1990 was Exosurf which contains DPPC, PG, and the stabilisers tyloxapol and hexadecanol. Another synthetic surfactant that has been used in both animal research and clinical trials is called artificial lung expanding compound (ALEC) which is a mixture of DPPC and phosphatidylglycerol in a ratio of 7:3 by weight.[65] The synthetic preparations do not contain the surfactant proteins. There are efforts currently underway, however, to develop engineered surfactants which contain synthetic phospholipids and recombinantly synthesised human surfactant proteins. The advantages of these surfactants are that they would not be from animal sources and they would contain the human surfactant proteins which may have important physiological roles in the function and metabolism of exogenously administered surfactant without the potential for antigenic stimulation of proteins derived from animal sources.

Animal studies of surfactant replacement therapy

Studies of SRT in animal models vary in their use of surfactant preparations, methods of administration, means of physiological assessment, and the species or the gestational age of the animals. This has created confusion in the field and makes comparison and interpretation of the studies very difficult. In general, studies which assess efficacy of surfactant preparations can be classified into three categories: (1) assessment of in vitro biophysical properties (surface activity, adsorption, and respreading abilities), (2) restoration of pulmonary function by surfactant preparations, and (3) in vivo physiological efficacy in premature animals. Detailed description and discussion of in vitro biophysical studies have been previously described and reviewed[64 66-78] and will not be discussed here.

RESTORATION OF PULMONARY FUNCTION BY SURFACTANT PREPARATIONS

Surfactant deficient model using excised lungs

The excised lavaged rat lung model, in which multiple lavages render the lung surfactant-deficient, provides a link between the in vitro biophysical data and the in vivo physiological

activity of surfactants.[79–82] Each lung acts as its own control and the efficacy of SRT is reflected by the short term improvement of quasistatic lung compliance measurements after lavage. Bermel and coworkers observed a progressive return to near normal quasistatic lung compliance in the surfactant-deficient model with increasing tracheal instillations of the modified natural surfactant, CLSE.[83] Ikegami and coworkers studied the ability of various phospholipids to restore lung pressure-volume characteristics in excised lavaged rat lungs.[84] Although various suspensions of DPPC and other phospholipids – for example, PG, phosphatidylinositol (PI), and phosphatidylserine (PS) – at a molar ratio of 9:1 had the ability to restore the pressure-volume characteristics toward normal, none was as effective as CLSE, suggesting that surfactant components other than the phospholipids were contributing to the overall functional activity of surfactant.[84]

Studies by McLean and coworkers used the excised lavaged rat lung model[80 81 85] and the in vivo lavaged guinea pig lung model[80 85] to test the efficacy of several formulations of synthetic peptides and phospholipids. Various peptides including a synthetic peptide based on residues 81–102 of SP-A, an analogue of SP-A with increased amphipathic α-helical potential, and the hydrophobic peptide gramicidin D were all ineffective as surfactant mixtures with DPPC in the excised lavaged rat lung model.[80] A later study tested three synthetic peptides, based on the putative amphipathic α-helical region of SP-A, as SRT in the same model.[81] The two peptides that corresponded to residues 81–102 and 78–101 of natural SP-A were ineffective. An analogue peptide with substitution of leucine residues for aspartate[84] and threonine[90] of $SP\text{-}A^{81-102}$ when mixed with DPPC, however, restored quasistatic lung compliance to 90% of the unlavaged value in the excised rat lung model.[81] In addition, McLean *et al*[80] demonstrated that a mixture of a lipid binding amphipathic α-helical peptide, 18-As (based on the lipid binding sequences of the plasma apolipoproteins), and DPPC was effective in restoring lung function in the isolated rat lung model of surfactant deficiency. The 18-As peptide sequence is different from SP-A and, in fact, mixtures of SP-A like peptides with DPPC were ineffective in restoring lung function in this model. These studies showed that SP-A, which is lacking in the modified natural surfactant preparations, may not be a necessary component of replacement surfactant in restoring short term quasistatic lung compliance in the excised rat lung and in vivo guinea pig lavaged lung models.

Recently, Bruni and coworkers studied the effects of synthetic peptides of SP-B and SP-C in combination with phospholipids and palmitic acid as replacement surfactants in the isolated rat lung model of surfactant deficiency.[86] These synthetic peptides were modified to increase α-helical structures and affinity for lipid layers (such as the oxidation of SP-B 1–78 by dimethylsulphoxide) and to enhance surface activity (such as the chemical acylation of SP-C 1–34). The synthetic peptides were shown to improve compliance by 10% in the surfactant-deficient isolated rat lung model. They concluded that synthetic surfactant peptides required biochemical modifications in order to assume secondary structures necessary for optimal function. Further studies may enable us to have a better understanding of the apoprotein-phospholipid interaction in the surfactant system, an important aspect for the success of SRT. Although these studies were successful in restoring compliance in these models, care must be taken in future evaluations using such synthetic peptides as they represent only fragments or parts of the entire complex and structural as well as conformational differences may exist which could complicate the interpretation of results. In conclusion, studies by McLean[80 81 85] and Bruni[86] on the surfactant proteins indicated that SP-B and SP-C might be critical as components of replacement surfactant in the excised rat lung model whereas SP-A might not.

Surfactant deficient model in vivo

The in vivo lavaged adult guinea pig lung as an experimental model for SRT was first reported by Lachmann.[87] In this model, alveolar surfactant phospholipids are removed by consecutive lung lavage and the short term survival of the animals is ensured by artificial ventilation. Biochemical, physiological, and histological findings after lavage were reported to mimic a pathological condition similar to the adult respiratory distress syndrome (ARDS) without causing severe damage to the alveolar structures.[87] Berggren and coworkers instilled modified natural surfactant from minced porcine lungs in the guinea pig lavaged lung model.[88] The surfactant-treated animals had significant improvement in gas exchange and alveolar air expansion, as shown by increased alveolar volume density on histological sections, when compared with a control group without treatment.

Adult rabbits subjected to lavage have also been used to study the effects of exogenous surfactant.[89–92] Kobayashi and coworkers investigated the effects of modified natural surfactant obtained from porcine lung lavage fluid in "lung lavaged" rabbits.[89] The survival rate, arterial blood gas tensions, and compliance of the group treated with both surfactant and positive end-expiratory pressure (PEEP) were significantly better than the groups which either received surfactant alone, PEEP alone, or no treatment at all. Oetomo and coworkers[90] administered natural sheep surfactant to rabbits that were lavaged four times to remove 80% of the alveolar phospholipids. Results showed that treated animals were able to re-establish spontaneous breathing and maintain normal arterial blood gases following SRT while all the control animals without treatment died during the weaning-off regimen in which the PEEP level was lowered from 2.5 to 0 cm H_2O. The same model has also been used for determining the effects of different forms of ventilation on hyaline membrane formation.[91 92] These studies compared conventional mechanical ventilation

with a PEEP of 1–2 mmHg (low) and PEEP equal to the inflection point pressure (high) in surfactant-deficient lavaged rabbits. Less lung damage was observed in the high PEEP group than those of the low PEEP group.[91 92] The authors concluded that high PEEP is advantageous in preventing alveolar collapse in neonatal RDS. These studies reaffirmed the usefulness of the lavaged rabbit lung model for testing the early effects of different surfactant preparations and ventilation patterns.

The benefit of the in vivo lavaged lung model over the in vitro excised lavaged lung model is the ability to detect a longer duration of effects of surfactant in living tissues. The disadvantage for both of these models, however, is the use of mature animals with fully developed lungs. These may not be relevant or applicable to neonatal HMD. Also, the capacity for surfactant regeneration in the mature lung may shorten the time course of recovery. Furthermore, the immature structure of the lung is a major factor in the pathogenesis of HMD. In addition, in order to have a meaningful determination of the efficacy of surfactant in these animal models, a standardised ventilation procedure is necessary because different ventilation manoeuvres may affect the outcome of the experiments.[91 92]

IN VIVO PHYSIOLOGICAL EFFICACY IN PREMATURE ANIMALS

Perhaps the most relevant animal model for surfactant deficiency in HMD is the use of premature animals in vivo.[93–118] Animals most commonly used are premature rabbits,[94–101 119–122] lambs,[102–111 118 123–130] and baboons.[112–117] It is more appropriate to extrapolate experimental findings from these preterm animals to the premature human infant.

Rabbits

Bronchiolar epithelial lesions that are similar to those in human HMD can be induced by artificial ventilation of 27 day gestation fetal rabbits (term is 31.5 days). Tracheal instillation of natural surfactant extracts from adult rabbits increase lung compliance and survival rate, and improves aeration of the lung parenchyma and resorption of fetal pulmonary fluid during spontaneous ventilation.[96 131]

A study in premature 27-day rabbits showed that significantly more radiolabelled albumin administered via the airways was recovered from the airspaces in the surfactant-treated animals than controls; on the other hand, significantly less radiolabelled albumin administered intravenously had permeated into the alveolar compartment of the surfactant-treated animals than control animals.[120] This indicated that SRT might improve lung function by reducing the leakage of surfactant-inhibiting serum proteins into the alveolar spaces of premature rabbits.

Oetomo and coworkers[90] studied the early effects of SRT on lung function and biochemical aspects using natural surfactant obtained from sheep lung lavage fluid. This surfactant improved survival when tested in vivo using immature rabbits and lambs with surfactant deficiency.[90] Comparative studies of the effects of artificial surfactant and natural surfactant on premature rabbit lungs have generally shown an advantage of natural surfactant.[111 121 122] Morley and coworkers found that DPPC:PG in a 7:3 molar ratio and natural surfactant extract from rabbit lungs were both effective in improving lung compliance although hyaline membranes were present in all groups.[121] A similar study compared DPPC:PG in various ratios with or without cholesterol or lyso-PC to natural surfactant extract from lavages of rabbit lungs.[122] Although the synthetic mixtures showed promising biophysical effects in vitro, the same effect could not be confirmed in vivo as the effects of artificial surfactant were inferior to those of the natural surfactant in terms of the restoration of pressure-volume characteristics in preterm rabbits.[122] Human surfactant, harvested from amniotic fluid, was given to premature rabbits and the treated animals showed improved chest excursion, decreased retractions of the rib cage during spontaneous breathing, and decreased death rate compared with control animals.[100]

Premature newborn rabbits were used to determine the relative efficacy of modified natural surfactant (Surfactant CK and Curosurf) versus apoprotein-based artificial surfactant.[97 132] The modified natural surfactants both contained the hydrophobic proteins SP-B and SP-C. Results showed that the artificial preparation was beneficial in restoring lung compliance and alveolar volume density, but to a lesser degree than the same dose of the modified natural surfactants. It is possible that other components of surfactant may have essential properties that improve the physiological effects which might be required to make the artificial material comparable to modified natural surfactant.

Recently, the effects of natural surfactant and surfactant constituted with specific apoproteins in preterm rabbits were evaluated.[101] Natural sheep surfactant was compared with synthetic surfactants containing the lipid components of surfactant to which SP-A, SP-B, or SP-C or combinations of the three apoproteins were added. Results showed that SP-A or SP-C, when added alone to surfactant lipids, failed to restore complete surfactant function in vivo.[101] Surfactant lipids with SP-B alone, however, improved dynamic lung compliance in premature rabbits. The effects of surfactant lipids with SP-B alone were similar to the constituted surfactant containing all three surfactant proteins. This indicated that SP-B was mainly responsible for enhanced activity. However, none of the constituted surfactants were as effective as natural sheep surfactant. The authors expressed the difficulty in correlating in vitro biophysical measurements to functional activity of surfactant in vivo. SP-B and SP-C differ in their ability to alter the adsorption and spreading characteristics of lipid mixtures in vitro. Furthermore, the same study showed that sheep surfactant extracts without PEEP demonstrated effective responses, whereas the synthetic surfactants containing surfactant pro-

teins without PEEP did not improve lung mechanics or protein leak responses.[101]

While the usefulness of the premature rabbit model in studies of SRT has been demonstrated, there are limitations to this model in that neither the effect of surfactant on gas exchange nor the duration of action was assessed. The period of ventilation possible in the preterm rabbit model is limited, allowing only the assessment of short term surfactant function.

Lambs

The need for other premature animal models that are conducive to long term ventilation prompted the use of lambs and baboons.[102-118 133] One advantage of premature lambs as an animal model for HMD is that births often occur as twins to allow experimental-control pairs for statistical analysis of data.[109] In this model the relative improvement in blood oxygenation, lung mechanics, and survival can be assessed.[110 111] Treatment of premature lambs with natural sheep surfactant demonstrated improvement in pulmonary function (pressure-volume curves) and gas exchange (arterial Po_2, Pco_2, and pH). The effect lasted for about eight hours in lambs treated at birth and only about three hours in lambs treated after the onset of respiratory failure.[111 118 123 128] CLSE also displayed excellent biophysical surface properties and had a pronounced effect on enhancing lung function in premature lambs in vivo.[134]

Jobe and coworkers investigated the kinetics of secretion of DPPC and the effects of natural sheep surfactant in lambs with or without pulmonary immaturity.[108] Lambs prematurely delivered by caesarean section and term lambs were supported on ventilators and studied over a period of two days. Radiolabelled palmitic acid was injected systemically after birth to detect the appearance of endogenously synthesised and secreted DPPC in sequential airway samples of preterm lambs treated with sheep surfactant, in untreated lambs, and in term lambs. Instillation of natural sheep surfactant in premature lambs resulted in improvements in lung compliance and gas exchange. The kinetics of release of DPPC into lavage fluid was linear and similar in both preterm and term lambs.

The quantity of natural surfactant necessary to prevent HMD in premature lambs was studied by Ikegami and coworkers.[124] Improvements in gas exchange and lung function in premature lambs receiving 19 mg/kg of natural sheep surfactant were similar to those receiving 53 mg/kg or more. The dose of surfactant (53 mg/kg) that resulted in good surface activity measurements in vitro was similar to other estimates of the amount of surfactant necessary to cover the alveolar surface. The authors concluded that the clinical response, rather than extrapolation from the dosage used in vitro, was the better way to evaluate the SRT.[124] On the other hand, Walther and coworkers showed that multiple doses of natural sheep surfactant at 50 mg/kg were more effective than a single initial dose administered after one hour of age to premature lambs with HMD, and the response to small, repetitive doses of surfactant was dependent on gestational age.[125] The onset of therapeutic action of bovine lipid extract surfactant in premature lambs was shown to occur within five minutes of surfactant instillation.[135] Major increases in functional residual capacity, vital capacity, and compliance took place within 5–20 minutes.

Ikegami and coworkers described the effects of natural sheep surfactant given to premature lambs on surface activity of lung lavage fluid.[127] Alveolar washes from the treated animals and the natural surfactant all had similar phospholipid compositions. However, the alveolar washes from the treated animals contained about 40 times more protein per micromole phosphatidylcholine than the natural sheep surfactant. Correspondingly, the surface activities of the alveolar washes of the treated animals were considerably lower than the sheep surfactant. This indicated that the natural surfactant was inactivated by an inhibitor of surfactant function such as serum proteins and/or other substances released from the vascular space.[127] Natural versus modified natural surfactant without SP-A were compared in a study of SRT on proteinaceous alveolar oedema in premature lamb lungs.[133] Intravascular radiolabelled albumin accumulation into premature lamb lungs was studied after SRT with either sheep surfactant or CLSE. The average net hourly albumin accumulation in the alveolar wash three hours after CLSE treatment was significantly higher than in those treated with sheep surfactant and not different from that in control lambs. However, by 24 hours the total protein in the alveolar washes of both surfactant-treated groups was similar to that measured in term newborn animals.

As in the premature rabbit model, different surfactant preparations have been tested for effectiveness in premature lambs. An artificial surfactant (a sonicated mixture of a 9:1 molar ratio of DPPC and PG) did not improve the lung compliance of unventilated premature lambs, whereas natural sheep surfactant resulted in improvement.[105] It was concluded that the surface tension properties of artificial surfactant were reversibly inhibited by fetal lung fluid whereas the natural sheep surfactant was affected to a much lesser extent.[105] CLSE was compared with an artificial surfactant composed of DPPC/PG in 7:3 molar ratio in terms of in vitro surface properties and in preterm lambs.[109] CLSE and DPPC/PG were able to lower surface tension to 1 dyne/cm under dynamic compression in vitro. However, the artificial surfactant did not improve lung compliance and arterial oxygenation. CLSE, on the other hand, improved the alveolar-arterial oxygen gradient, blood gas tensions, and lung compliance. A similar study compared natural sheep surfactant with an artificial surfactant composed of DPPC/PG in a 9:1 molar ratio in premature lambs.[126] The artificial surfactant was not effective in restoring the pressure-volume characteristics of previously unventilated lamb lung, whereas the natural sur-

factant was.[126] The authors speculated that the poor response may be unique to the immature lung and suggested a new approach to the design of other artificial surfactants.

Cummings and coworkers reported that natural sheep surfactant, CLSE, and Survanta, but not Exosurf, improved oxygenation, lung mechanics, and survival in the premature lamb.[111] This is in contrast to a previous study by Durand *et al*[130] in which Exosurf was reported to be as effective as sheep surfactant in significantly improving the survival and pulmonary function in preterm lambs. Differences in methodology and prematurity of the lambs may explain the disparity in results. Cummings administered surfactant to 126-day lambs immediately after umbilical cord ligation, whereas Durand administered surfactant to 132-day lambs before cord ligation.[111 130]

A recent study of preterm lambs confirmed the better response in gas exchange and compliance to Survanta compared with synthetic, protein-free surfactant.[136] After five hours of ventilation, large and small aggregate surfactant fractions were isolated from the bronchoalveolar lavage (BAL) fluid of the Survanta-treated animals. The large aggregate fraction had the most surface activity and contained SP-A, SP-B, and SP-C. The small aggregates were ineffective as surfactants. BAL fluid from Survanta-treated lambs contained more SP-A than did BAL fluid from the lambs treated with the other surfactants. The surfactant recovered from preterm lambs increased compliance in surfactant-deficient immature rabbits. The large aggregate fractions which contain both the exogenous and endogenous surfactant pools in the BAL fluid from lambs treated with Survanta improved compliance in the preterm rabbits to a greater extent than did the original surfactants which contain only exogenous surfactant. Thus, exogenous surfactant may gain increased effectiveness from interaction with the endogenous pool of surfactant. The surfactant proteins appear to play an important role in this interaction in vivo.

Baboons

Premature baboons have been used as an alternative model of HMD and bronchopulmonary dysplasia (BPD).[112–117] Two studies examined histological changes in the lungs of seven baboons delivered prematurely (75% of full gestation) and one at term which were mechanically ventilated and exposed to high levels of inspired oxygen.[112 113] Six of the seven premature baboons developed pathological lesions of HMD and/or BPD. The histopathological features of the premature baboon model are similar to those of BPD in the human neonate.[112 113] Several studies have used the baboon model for the investigation of the prevention and treatment of HMD using surfactant.[114–116]

Surfactant TA (100 mg/kg), when administered to the premature baboon at 10 minutes of age, significantly improved the arterial/alveolar oxygen ratio and pulmonary compliance, facilitated rapid weaning from assisted ventilation, and lung histological examination revealed no signs of early BPD.[114] The saline control group revealed many features of early BPD such as dysplastic maturation, air trapping, or cellular atypia.[114] Another study investigated the difference in clinical responses to early Surfactant TA treatment in the two HMD models (lambs in two groups at 125 and 132 days gestational age and baboons at 140 days gestational age).[115] Term gestations for the lamb and baboon are 148 days and 187 days, respectively. The surfactant-treated baboons (100 mg/kg) were shown to have a sustained and significant improvement in arterial Po_2 from the time of instillation to eight hours after treatment. The surfactant-treated lambs of 125 days gestation showed a good but transient response with an increase in the arterial/alveolar oxygen ratio and a decrease in mean airway pressure. No significant improvement in oxygenation was found among the treated and untreated 132-day lamb groups due to their advanced maturity and larger variance. The authors concluded that the differences in response to the same surfactant therapy among the lamb and baboon models were due to differences in species, lung maturation, and the differences in alveolar protein leakage.[115]

Investigators also studied the differences in early (10 minutes of age) and late (two hours of age) treatments using Surfactant TA in the baboon model of HMD.[116] Both treated groups had significantly higher compliance and arterial/alveolar oxygen ratio and lower mean airway pressure and oxygen requirements than the untreated group. However, early surfactant treatment resulted in a greater improvement in lung mechanics than did late treatment.

CORRELATION OF IN VITRO SURFACE PROPERTIES AND PHYSIOLOGICAL RESPONSE

Recent evidence suggests that in vitro biophysical properties may not be important or may not predict in vivo response to surfactant. Correlation of in vitro surface properties and responses of preterm lambs to SRT was studied using four different surfactants: natural sheep surfactant, natural rabbit surfactant, natural human surfactant, and Surfactant TA.[137] While fourfold less Surfactant TA was required to lower the minimum surface tension below 10 dyne/cm than for the other surfactants, Surfactant TA in preterm lambs was less effective than sheep surfactant in terms of improved gas exchange. The in vivo responses to rabbit surfactant were intermediate between the responses to sheep surfactant and to Surfactant TA. Human surfactant was the least effective surfactant in vivo in the study, possibly due to a problem of batch-to-batch variability. The study demonstrated that the range of clinical responses was not predictable based on the in vitro surface properties.[137]

Another investigation showed that three different surfactants effectively improved compliance and enhanced alveolar volume density in histological sections in preterm rabbits despite wide variations in surface properties.[138] Surfactants tested were (1) natural surfactant

extract from minced bovine lungs with rapid respreading and a relatively high minimal surface tension during surface compression; (2) bovine surfactant enriched with DPPC, tripalmitin, and palmitic acid with slow spreading and low minimal surface tension; and (3) bovine surfactant enriched with DPPC and dipalmitin with rapid spreading and low minimal surface tension. All three preparations were effective in vivo, illustrating that in vitro surface properties do not seem to influence the outcome of in vivo efficacy.[138]

In order to determine the specific surfactant properties necessary for improving gas exchange in premature animals, Tween 20 (a detergent of high surface tension and poor elasticity or compressibility as compared with natural surfactant) was tested in premature lambs.[3] Tween 20 significantly improved gas exchange and lung compliance in preterm lambs. Another study showed that agents lacking the minimum surface tension and hysteresis of natural surfactant (such as the fluorocarbon FC-100 and Tween 20) were as effective as natural surfactant in improving lung expansion, gas exchange, and compliance in preterm lambs with respiratory failure.[4] Taken together, these studies[3 4 137 138] indicate that surface activity may not be as important in improving gas exchange and lung compliance as previously thought. The anti-stick or lubricant properties of surfactant may play an important part.

STRUCTURE-FUNCTION RELATIONSHIPS OF SURFACTANT COMPONENTS

While the previously mentioned excised lavaged rat lung model and the in vivo lavaged lung model are adequate in generating initial physicochemical and physiological data of surfactant preparations, it is important to directly correlate in vitro surface activities with physiological activities in vivo. Tanaka and coworkers attempted to correlate surface activities, chemical composition, and physiological effects of Surfactant TA using the Wilhelmy balance, excised lavaged rat lung, and premature rabbits.[139 140] The investigators found that the content of DPPC in the Surfactant TA was the important determinant of minimum surface tension, the spreading rate, and pressure-volume characteristics in vitro and in vivo.[139] Lung surfactant extract from minced bovine lung was supplemented with different kinds and amounts of fatty acids and triacylglycerols and their surface properties were tested in the excised lavaged rat lung model. Lung surfactants modified with palmitic acid-tripalmitoylglycerol and stearic acid-tristearoylglycerol gave better lung pressure-volume characteristics in the excised lavaged rat lung than those with oleic acid-triacylglycerols. The results showed that improved surface activity and physiological restoration of lung compliance were associated with surfactant that contained stearic acid, palmitic acid, and the hydrophobic surfactant proteins. Also, the fatty acids gave better surface activity than triacylglycerols.[140]

While it is important to correlate in vitro and in vivo activities of various surfactant preparations, it is also critical to determine the specific surfactant components that are responsible for the corresponding surfactant properties in vivo. Several recent studies have attempted to correlate surfactant phospholipid component structure with functional activities. Tanaka and coworkers prepared a series of 25 synthetic lung surfactant mixtures and tested the in vitro biophysical properties and physiological activities in premature rabbits.[141] DPPC, the surfactant apoproteins, palmitic or stearic acid, and PG or phosphatidylserine all were shown to be important surfactant components for both in vitro and in vivo activities. Restoration of the compliance of excised rat lung with various phospholipids was investigated by Ikegami and coworkers.[142] Treatment with 9:1 mixtures of DPPC with various other phospholipids – for example, DPPG, unsaturated phosphatidylinositol, unsaturated phosphatidylserine, PG, and lecithin – showed that DPPC:PG produced the most improvement in lung compliance but none of the phospholipid mixtures was as effective as natural surfactant.

Surfactant analogues were synthesised as probes for structure-activity studies as well as pharmacological tools to investigate phospholipid-surfactant protein interactions. Turcotte and coworkers synthesised and characterised a series of surfactant analogues structurally similar to the DPPC and other major glycerophospholipids in the surfactant system.[66 74 75 78] These surfactant analogues were designed to be non-substrates for the phospholipases A_1 (E.C. 3.1.1.32), A_2 (E.C. 3.1.1.4), and D (E.C. 3.1.4.4). These analogues are more hydrophobic than DPPC. They demonstrated enhanced respreading facility in surface films on a Wilhelmy balance without any loss in dynamic surface activity (minimum surface tension of <1 mN/m on Wilhelmy balance, oscillating bubble). Several analogues also had better adsorption than DPPC, although none approached the optimal behaviour of natural surfactant. The encouraging physical data suggested that these analogues may be useful probes for structural-activity relationships relevant to pulmonary surfactant phospholipids. The first analogue to be tested was dimethyl(3-phosphonopropyl) ammonium, mono(2,3-bis-(hexadecyloxy)-propyl)ester (DEPN-8), a diether phosphonate analogue of DPPC.[66 74 78] DEPN-8 had better surface activity than DPPC, including enhanced respreading facility in surface films on a Wilhelmy balance without any loss in dynamic surface tension lowering ability (minimums of <1 mN/m on Wilhelmy balance, oscillating bubble). The analogue also had improved adsorption over DPPC, although it did not approach the optimal behaviour of natural surfactant. DEPN-8 partially restored lung compliance in surfactant-deficient excised rat lungs.[66] DEPN-8, administered as a single agent in saline suspension (50 and 25 mg/rat lung), improved compliance in the excised rat lung significantly more than an equivalent amount of DPPC. The in vivo function of surfactants containing DEPN-8 was also investigated in preterm rabbits.[143] DEPN-8 was

Table 3 Efficacy of surfactant preparations in animal studies

Surfactants	*Biophysical effects*	*Lavaged lung*		*Premature animals*		
		In vitro	*In vivo*	*Rabbit*	*Lamb*	*Baboon*
Natural						
Sheep	↑123 137	↑142		↑101 120	↑102 104	
Human	↑100			↑100	↑129	
Modified natural						
CLSE	↑68 69 73 134	↑83	↑90		↑109–111 134	
Survanta	↑138			↑136 138	↑111 136	
Surfactant TA	↑137 139	↑139		↑139	↑115	↑114 115
Synthetics						
Exosurf				↓136	↑130 ↓111	
DPPC/PG (7:3)	↑109	↑84		↑105 121	↑109 126 134	
DPPC/PG (9:1)	↑79	↑79 105			↓105 126	
α-helical peptides	↑80	↑81	↑80 85			
Surfactant analogues	↑66 74 75 78	↑74		↑143		

↑ Significant improvement versus control.
↓ Insignificant improvement versus other preparations or control.

shown to be as effective as DPPC as lipid components of synthetic surfactants. Also, in a similar manner to DPPC, the analogue DEPN-8 interacted with SP-B and SP-C and improved in vivo function to levels comparable to natural sheep surfactant. Future studies of similar classes of surfactant analogues mixed with neutral lipids and/or proteins may further define the molecular components of natural surfactant that are critical for its specific surface active properties. These analogues can also be used as probes to study correlations between physicochemical and physiological activity. These probes may also further define the molecular components of natural surfactant that are critical for its functional activity. This may eventually guide the development of new surface-active molecules for potential improvement of synthetic surfactant replacement mixtures.

EFFICACY OF SRT IN ANIMAL MODELS OF ARDS

Bovine lipid extract surfactant was used therapeutically in various animal models of ARDS.[144–146] A mouse model for influenza A virus and a rat model for Sendai virus were used to induce viral pneumonia with acute disease closely resembling ARDS.[144] The reduced arterial oxygenation and pulmonary compliance caused by the loss of surfactant function during viral pneumonia were almost completely restored by SRT using bovine lipid extract surfactant. Bovine surfactant was also shown to improve pulmonary function and gas exchange in a rat model for *Pneumocystis carinii* pneumonia.[145 146] These findings indicated the usefulness of SRT for the treatment of respiratory failure due to severe viral pneumonia.

Engstrom and coworkers reported that CLSE increased 72 hour survival of rabbits that were exposed to 100% oxygen by attenuating the increase in lung protein permeability usually found in hyperoxia-induced lung injury.[147] Curosurf, however, did not improve the blood gases, nor the compliance at a late stage (85–88 hours) of hyperoxia-exposed guinea pigs, possibly due to the presence of inhibitory components in the lung oedema fluid.[148] These results indicated that surfactant might be more effective at an earlier stage of the disease. There was a concentration-dependent inhibition of surfactant function by the lung oedema fluid from animals exposed to hyperoxia.[149] Survanta reduced the recoil of both excised lung and lungs in vivo acutely injured by acid aspiration, but did not improve gas exchange.[150] The authors related their results to those of Jacobs *et al* with Tween-20 mentioned previously, further demonstrating that the properties of surfactant thought to be responsible for improving lung compliance were quite different from those contributing to improved gas exchange. Sheep surfactant was shown to improve the arterial blood gas values and lung compliances in acute lung injury (as characterised by pulmonary oedema, decreased lung compliance, and atelectasis) induced by bilateral cervical vagotomy in adult rabbits.[151] Again the exogenous surfactant was thought to overcome in part the detrimental effects of inhibitory plasma proteins in pulmonary oedema. In addition, bovine surfactant significantly improved blood gases and lung mechanics in guinea pigs with acute lung injury induced by intravenous injection of an anti-lung serum.[152] Thus, the effects of SRT in various ARDS models was beneficial but dependent on the type of animal model, the degree of lung injury at the time of treatment, and the initiation of treatment.

Table 3 summarises the efficacy of various surfactant preparations as reported by the specific studies. The results of these studies can be classified into two categories: (1) significant improvement with treatment, or (2) insignificant improvement compared with other preparations of surfactant or control.

Clinical studies of neonatal HMD

Initial studies using tracheal instillation or nebulised administration of a synthetic mixture of DPPC and PG did not show any effect on the clinical course or gas exchange in infants with HMD.[153–155] These trials, using an aqueous mixture of these phospholipids sometimes referred to as artificial lung expanding compound (ALEC), did not include agents that would improve the adsorption and spreadability of these preparations over the alveolar surface. The first successful attempt to improve the condition of infants suffering from HMD was by Fujiwara and his associates using modified natural bovine surfactant extract in an uncontrolled pilot study of 10 infants suffering from HMD[156] in whom an improvement in oxygenation and a decreased need for mechanical ventilation was reported. Since then numerous randomised controlled clinical trials have been performed documenting the efficacy and safety of exogenous SRT in neonates suffering from HMD. Two strategies have been used in these studies: a rescue strategy in which infants with documented HMD are then treated with SRT, and a prophylactic approach in which all eligible infants at risk for developing HMD are treated either at the time of birth or as soon as possible thereafter.

RESCUE STUDIES

There have been many studies showing that SRT of infants suffering from HMD results in

significant improvements in gas exchange (as measured by oxygenation index, blood gas analysis) and ventilator settings, such as mean airway pressure, F_{IO_2}, and peak inspiratory pressure.[157-172] Most studies have also shown a reduction in the incidence of pulmonary air leak – that is, pneumothorax, pulmonary interstitial emphysema, pneumomediastinum and pneumopericardium – as well as improved survival of infants treated with SRT. Unfortunately, none of the studies have shown a consistent effect on the incidence of chronic lung disease (bronchopulmonary dysplasia), duration of hospital stay, duration of mechanical ventilation, or other neonatal problems including patent ductus arteriosus (PDA), intraventricular haemorrhage (IVH), and necrotising enterocolitis. One prospective randomised controlled multicentre trial did document an increased incidence of IVH[162] and another study showed an increase in the incidence of PDA[157] in infants treated with surfactant, but neither of these findings have been confirmed by other studies.

Modified natural surfactant

In a double blind, randomised, controlled trial Surfactant TA treatment of preterm infants suffering from HMD with birth weights of 750–1750 g improved oxygenation and ventilation, reduced the incidence of pneumothorax and pulmonary interstitial emphysema, but was associated with an increased incidence of PDA.[157] Survival without bronchopulmonary dysplasia (BPD) was associated with SRT. Gitlin and colleagues found that treatment with Surfactant TA of infants of birth weights 1000–1500 g with HMD improved oxygenation and ventilation within four hours of treatment.[158] Treatment was associated with fewer pneumothoraces and there were no adverse effects. There were no effects on the occurrence of PDA, IVH, BPD, or mortality; 11% of infants did not respond to treatment. Fujiwara and coworkers reported that treatment with single doses of Surfactant TA of infants with HMD weighing 750–1750 g enrolled in a multicentre randomised controlled trial reduced the severity of HMD, reduced the incidence of pneumothorax, pulmonary interstitial emphysema, and IVH, and improved survival without BPD.[159] There were no effects of SRT on the incidences of PDA, pulmonary haemorrhage, or mortality.

A randomised controlled trial comparing single versus multiple doses of CLSE for infants 30–36 weeks gestation with HMD showed that surfactant improved oxygenation rapidly within 10 minutes, and the effect lasted up to 48 hours in both single and multiple dose groups.[160] Approximately 70–80% of infants had transient responses and met criteria for retreatment.[160] There were no differences in the short term complications or long term outcome.

Randomised controlled trials of Survanta treatment of HMD in infants weighing 750–1750 g revealed that surfactant improved oxygenation, reduced mean airway pressure needed for mechanical ventilation, and reduced the incidence of pulmonary air leaks.[161,162] There were no effects on the incidences of PDA, BPD or necrotising enterocolitis, but one study showed an alarming increase in the incidence of IVH associated with SRT.[162] Multiple doses of Survanta therapy of HMD in infants weighing 600–1750 g resulted in improvements in oxygenation and ventilation, reduction of pulmonary air leaks and mortality, and improved survival without BPD.[163] There were no effects on the incidences of PDA, necrotising enterocolitis, IVH, or pulmonary haemorrhage.

Treatment of infants with HMD weighing 700–2000 g using porcine surfactant extract (Curosurf) resulted in improved oxygenation and ventilation, reduced pulmonary air leak, reduced mortality, and decreased incidence of BPD.[164-166] Multiple doses of Curosurf were more effective than single doses in the reduction of the incidences of pneumothorax and neonatal mortality.[167]

Natural human surfactant treatment of HMD in infants of 24–32 weeks gestation improved oxygenation and ventilation, reduced the incidence of pulmonary air leak, and improved survival without BPD.[168,169] The improvement in survival was associated with increased birth weight, earlier age at treatment, and female gender.[169]

Approximately 21% of infants treated with surfactant will have a transient response and between 10% and 30% of infants will have no response to SRT.[160,173] Several factors have been shown to influence the response to SRT in babies with HMD. The response to treatment is attenuated by several factors including asphyxia, male sex, and severity of the disease at the time of treatment.[173,174] In addition, response to treatment may be improved by early treatment within three hours of birth compared with later treatment at 15 hours of age.[175] Thus, it is possible that such differences in factors affecting the response to treatment may account for the lack of a consistent improvement in long term outcome measures such as survival and incidence of BPD.

Administration of surfactant in two fractional doses through a suction valve that did not require removal of the infant from the ventilator was as effective as administration using either two or four fractional doses given after removal from the ventilator.[176] Thus, SRT can be given to an infant without interrupting mechanical ventilation which results in fewer episodes of reflux of surfactant into the endotracheal tube and fewer episodes of oxyhaemoglobin desaturation during administration.[176]

Several studies have shown that exogenous SRT is not associated with enhanced lung inflammation although there is an increase in the number of alveolar macrophages recovered from bronchoalveolar lavage fluid in the third to seventh days of life.[177,178] Since the modified natural surfactant preparations contain the hydrophobic surfactant proteins, there is a potential for antigenic stimulation of infants treated with these preparations. Infants suffering from HMD have more permeable pulmonary capillaries which may lead to ab-

sorption of surfactant proteins into their bloodstream, thus exposing them to these antigens. Surfactant therapy was associated with a decrease in the transient rise of IgM antibody to surfactant proteins, SP-A, SP-B and SP-C,[179] suggesting that such therapy may reduce the leak of surfactant proteins into the vascular space by reducing lung damage. Another study showed that none of the infants treated with modified natural surfactants developed antibodies to SP-B or C after they had recovered.[180]

Compared with controls, there is no difference in long term developmental outcome of infants treated with surfactant as measured by the Bayley mental or motor scale at 12 and 24 months of age.[181] SRT has been shown to be cost effective by improving survival without increasing overall hospital costs. Total hospital charges to produce a surviving infant were over US$18 000 less in infants treated with SRT in one study.[182]

A Medline search of the English literature of all randomised controlled trials of surfactant therapy used in infants was performed to conduct a meta-analysis. Studies were grouped into five categories: rescue therapy with modified natural surfactant, rescue therapy with synthetic surfactant, prophylaxis with modified natural surfactant, prophylaxis with synthetic surfactant, and rescue therapy versus prophylaxis with modified natural surfactant. We determined the relative risk ratio for each study (proportion of treated infants with the outcome variable/proportion of control infants with the outcome variable), and mean ±95% confidence intervals (CI) were calculated for each risk ratio. Risk ratios in which the 95% CI do not include 1 indicate a significant change in the risk for that variable, a relative risk ratio of <1 indicates a reduced risk for that variable, and a risk ratio of >1 signifies an increased risk for that variable.

A meta-analysis of modified natural surfactant extracts used in rescue therapy of infants with HMD weighing 700–2000 g is shown in table 4 and fig 1A. There was a significant decrease in the incidence of pneumothorax associated with SRT as well as a significant decrease in mortality. Significantly more infants survived without chronic lung disease if they had been treated with SRT using the modified natural surfactants. There was no effective change in the incidence of BPD.

Two studies of SRT in full term newborn infants have been reported. In a prospective uncontrolled trial using calf lung surfactant extract in full term infants suffering from a variety of causes of respiratory failure surfactant treatment was associated with a significant improvement in oxygenation.[183] This study indicated that surfactant supplementation may be of benefit in newborn infants with respiratory failure due to either pneumonia or meconium aspiration syndrome. Unfortunately, no prospective randomised controlled trial of surfactant in this population has been performed. In an uncontrolled retrospective series SRT was associated with an improvement in oxygenation in full term infants with respiratory failure due to RDS.[184] There was no significant response in terms of oxygenation of infants with meconium aspiration syndrome treated with surfactant. At this time it appears that a randomised controlled trial of SRT in full term infants is warranted.

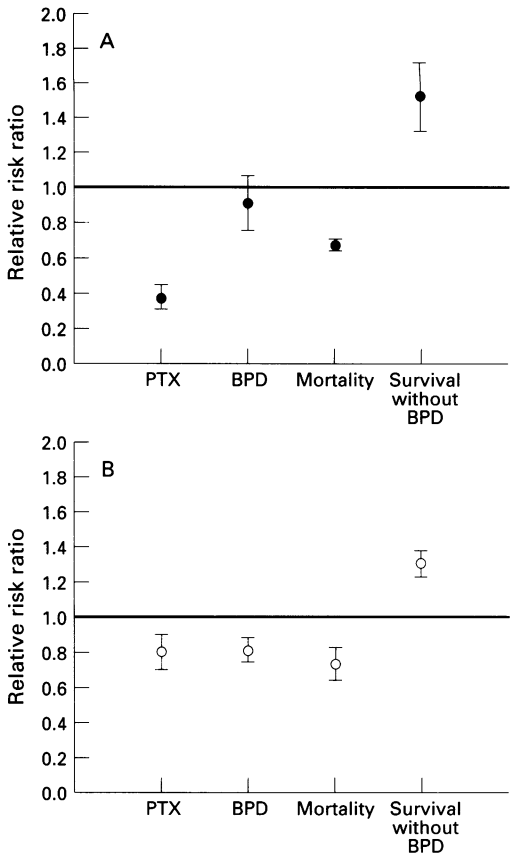

Figure 1 Meta-analysis of rescue trials using (A) modified natural surfactant and (B) synthetic surfactant. Data represent the mean ±95% confidence intervals for relative risk ratios for pneumothorax (PTX), bronchopulmonary dysplasia (BPD), mortality, and survival without BPD. Confidence intervals which do not include the risk ratio of 1 indicate significant reductions (<1) or increases (>1) in the chances for a particular outcome variable.

Table 4 Meta-analysis of rescue studies of surfactant therapy

	Modified natural surfactant		*Synthetic surfactant*	
	Control	*Treated*	*Control*	*Treated*
Pneumothorax	41.9 (5)	16.4 (3.8)	34.7 (6.1)	28.9 (7.8)
BPD	29.4 (3.8)	25 (4.6)	35.5 (12.1)	28 (8.1)
Mortality	31 (4)	20.5 (2.3)	30.6 (7.8)	23.5 (7.6)
Survival without BPD	42.2 (5.7)	60.8 (5.6)	44.6 (9.2)	56.5 (9.7)

BPD = bronchopulmonary dysplasia.

Synthetic surfactant

Studies with Exosurf in the rescue of infants suffering from HMD have shown that 35–40% of infants treated show a transient response or no response at all.[170] Exosurf treatment of HMD in infants weighing >650 g resulted in improved oxygenation and ventilation,[170] but 36% of infants had a transient response to therapy. Exosurf therapy of infants with HMD weighing >1250 g reduced the incidences of pneumothorax, IVH, and PDA, improved oxygenation and ventilation, improved survival,

and reduced the incidence of BPD.[171] Infants weighing 700–1350 g treated with Exosurf had improved oxygenation and ventilation, reduced pulmonary air leaks, and improved survival.[172] The incidence of BPD was not affected although survival without BPD was improved.[172] Thus, most studies of synthetic surfactant used for rescue therapy have shown improvement in oxygenation and decreased need for mechanical ventilation.[170–172 185 186] This improvement in oxygenation occurs about six hours later than that produced with modified natural surfactant.[187] Although these acute effects on oxygenation and ventilation appear to favour modified natural surfactants, the incidence of chronic lung disease and survival without chronic lung disease are not different between these preparations.

There have been several studies comparing early and late rescue SRT. The OSIRIS Collaborative Group in Europe conducted the largest multicentre randomised trial ever performed in neonatal medicine.[188] They compared administration of Exosurf at a mean of 118 minutes after birth with administration at approximately three hours after birth. The infants receiving earlier treatment had significantly fewer pneumothoraces and a significantly greater number survived without chronic lung disease.[188] Interestingly, the study showed that it was unlikely that the third and fourth doses of Exosurf had any important clinical benefit when compared with the effects of the first two doses.

Treatment of infants with HMD using synthetic surfactant results in a decrease in pulmonary artery pressure and an increase in blood flow through the ductus arteriosus.[189 190] Synthetic SRT used in the treatment of infants with HMD has been shown to decrease airway resistance and decrease the work of breathing in these infants.[191] Long term follow up has shown that, compared with controls who did not receive Exosurf, infants treated with synthetic surfactant have decreased airway resistance and decreased work of breathing as early as three months of age, and this effect persists to at least 12 months of life.[191] In a non-randomised trial, Exosurf therapy was associated with a 35% increase in cerebral blood flow.[192] This transient increase in blood flow velocity to the brain may place these infants at risk for intraventricular haemorrhage which might explain the lack of a consistent reduction in the incidence or severity of intracranial haemorrhages in infants treated with SRT.

One study showed that Exosurf resulted in enhanced mucociliary clearance of respiratory secretions.[193] This was associated with less viscous mucus obtained from infants treated with synthetic surfactant.[193] Thus, it is possible that synthetic surfactant may improve oxygenation and ventilation in infants in part by improving the quality of mucus in the respiratory tree, making it less viscous and better transported and cleared.

A meta-analysis of the studies involving synthetic surfactant in the rescue of infants suffering from HMD is shown in table 4 and fig 1B. There is a consistent decrease in the incidence of air leaks and a decrease in both mortality and the incidence of BPD in infants treated with synthetic surfactant. The reduction in the relative risk of pneumothorax (relative risk 0.8) is not as prominent as that associated with treatment using modified natural surfactant (relative risk 0.4). Similar to modified natural surfactant, synthetic surfactant treatment enhances survival of infants without BPD.

Which preparation of surfactant is more efficacious? A recent randomised controlled study compared the efficacy of modified natural surfactant (Survanta) with synthetic surfactant (Exosurf).[194] Infants receiving modified natural surfactant had a more rapid improvement in oxygenation and ventilation, but there were no differences in long term outcome variables such as the development of BPD, mortality, and survival without BPD. The potential benefit of more rapid improvements in gas exchange with concomitant weaning of ventilator support did not result in improved long term outcome. This makes recommendations of one preparation over another difficult. The choice of which preparation to use is a matter of individual preference until further comparative studies are published.

PROPHYLAXIS STUDIES

Modified natural surfactant

There have been several studies using modified natural surfactant for the prevention of HMD in infants of <30 weeks gestation. Administration of CLSE to infants of <30 weeks gestation prior to the first breath significantly decreases the incidence of RDS from 64% (control group) to 36% (treated group) and results in a significant improvement in ventilatory index and oxygenation[195] associated with a decrease in the need for supplemental oxygen and mean airway pressure used to ventilate the infants.[196] In addition, there are significantly fewer air leaks (pneumothorax and pulmonary interstitial emphysema) and an increase in survival in the infants treated with surfactant. Prophylaxis with CLSE in infants of 25–29 weeks gestational age also results in a decrease in the severity of disease.[197] Prophylactic treatment of infants of 24–30 weeks gestational age with Survanta is associated with decreased incidence of RDS, less severe radiographic changes on the chest radiograph, decreased incidence of pneumothorax, and improved survival without chronic lung disease.[198] Prophylaxis with Survanta reduced the incidence of HMD, improved oxygenation and ventilation, reduced the risk of pulmonary air leaks, improved survival, and improved survival without BPD in infants of 23–29 weeks gestation (weighing 600–1250 g).[199] These effects were most pronounced in the infants weighing <1000 g. A multicentre randomised controlled trial of bovine surfactant in Europe also documented an improvement in survival without chronic lung disease (from 40% to 76%) in infants of 25–30 weeks gestation receiving prophylaxis.[200]

Table 5 Meta-analysis of prophylaxis studies of surfactant therapy with modified natural surfactant

	Control	Treated
Incidence of HMD	58.1 (3)	28.2 (3.7)
Pneumothorax	24.1 (5.8)	12.2 (5.4)
BPD	45.5 (7.6)	33.6 (7.3)
Survival	76.5 (5.4)	91 (2.3)
Survival without BPD	35.2 (7.6)	64.2 (5.5)

HMD = hyaline membrane disease; BPD = bronchopulmonary dysplasia.

Natural human surfactant for the prevention of HMD significantly improved gas exchange, survival, and survival of infants without chronic lung disease.[201] The surviving surfactant treated infants spent almost two months less time in a neonatal intensive care unit than control infants.[201]

Overall, most of the studies using natural or modified natural surfactant to prevent HMD have shown a reduction in the incidence of HMD, improved survival, and decreased incidence of BPD in infants of <26 weeks gestation or <1000 g birth weight. Prophylactic therapy is associated with improved gas exchange as well as a decrease in air leaks. The variations in efficacy between studies are probably due to differences in strategy in ventilation as well as the differences in preparations and time of administration. A recent study has shown that a ventilator strategy using higher mean airway pressures and lower supplemental oxygen concentrations may be associated with better efficacy of prophylactic human SRT.[202]

A meta-analysis of the prophylactic studies using modified natural surfactant is shown in table 5 and fig 2A. Overall, the incidence of RDS has been significantly decreased by 50%. There is a decrease in the risk for pneumothorax and BPD and an increase in survival. The number of survivors without BPD is also significantly improved with prophylactic therapy. About 30% of infants treated prophylactically with surfactant preparations, however, develop HMD.

Synthetic surfactant

Exosurf. Prophylactic therapy with Exosurf does not reduce the incidence of RDS.[203,204] Synthetic surfactant treatment within one hour of birth of infants weighing 700–1350 g improves gas exchange, reduces ventilator settings with lower mean airway pressures, reduces the incidence of air leak, and improves survival without BPD compared with controls.[170,203,204] There are no significant effects on the occurrence of pneumothorax, intracranial haemorrhage, chronic lung disease, or mortality. Apgar scores at both one and five minutes were lower in the treatment group in one study.[170]

As shown in table 6 and fig 2B, meta-analysis reveals that prophylactic synthetic surfactant does not prevent HMD. The incidence of HMD in both treated and control infants remains about 50%, and there is no reduction in the relative risk of HMD (fig 2B). The relative risk of pneumothorax is reduced by 40% using synthetic surfactant as prophylaxis, and there is an increase in survival as well as survival without chronic lung disease (fig 2B). Synthetic surfactant used prophylactically has no effect on the development of BPD (table 6, fig 2B). As shown in fig 2, the efficacy of modified natural surfactant appears greater than synthetic surfactant in each of the outcomes analysed. Overall, there appears to be a significant decrease in the Apgar score at one minute, with one study showing a decrease in the Apgar score at both one and five minutes which raises the question of whether this could lead to significant adverse effects in the long term. Since SRT has been associated with cerebroelectrical depression[205] as well as significant changes in cerebral blood flow,[192] this could predispose to adverse outcomes both in the short term with intraventricular haemorrhage and in long term neurodevelopmental sequelae. These potential adverse effects may explain why there is not a decrease in intraventricular

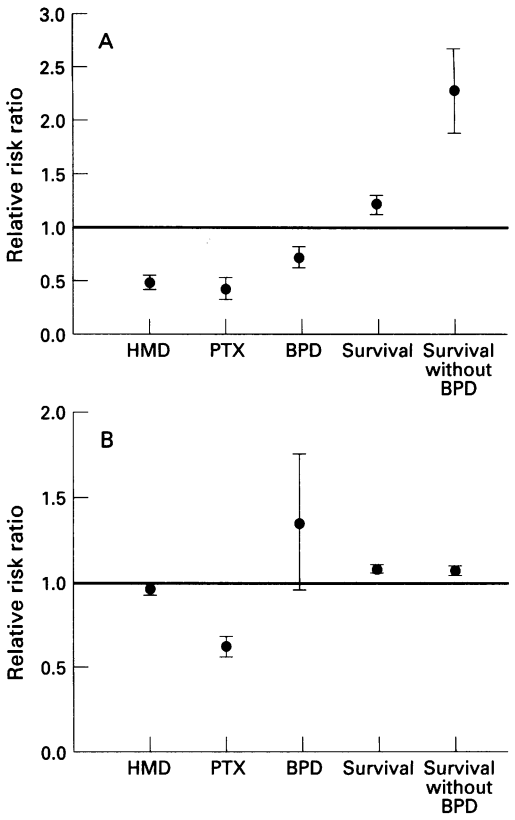

Figure 2 Meta-analysis of prophylaxis trials using (A) modified natural surfactant and (B) synthetic surfactant. Data represent the mean ± 95% confidence intervals for relative risk ratios for the incidence of hyaline membrane disease (HMD), pneumothorax (PTX), bronchopulmonary dysplasia (BPD), survival, and survival without BPD. Confidence intervals which do not include the risk ratio of 1 indicate significant reductions (<1) or increases (>1) in the chances for a particular outcome variable.

Table 6 Meta-analysis of prophylaxis studies of surfactant therapy with synthetic surfactant

	Control	Treated
Incidence of HMD	50.3 (4.7)	48.3 (6.6)
Pneumothorax	32.1 (10.1)	19.5 (6.1)
BPD	15.1 (5.3)	16.3 (2.7)
Survival	82.1 (3.5)	89 (2.4)
Survival without BPD	69.1 (2.7)	74.2 (2.4)

HMD = hyaline membrane disease; BPD = bronchopulmonary dysplasia.

haemorrhage associated with the decrease in the incidence and severity of HMD following treatment with SRT. This is certainly an area for future study.

Artificial lung expanding compound (ALEC). Two randomised controlled clinical trials of ALEC have been conducted. In a study in infants of 23–34 weeks gestation[206] only infants of less than 30 weeks gestation treated with ALEC had improved gas exchange (lower oxygen requirements), improved compliance (lower average peak inspiratory pressures), and reduced mortality. These infants also had fewer intraventricular haemorrhages and fewer infants in the treated group required oxygen for more than 28 days. There was no effect on the incidence of pneumothorax. Another multicentre trial of ALEC given at birth to infants of 25–29 weeks gestation, which included the data from infants of 25–29 weeks gestation in the first study, showed improved survival in the treated infants.[207] Thirty percent of the control infants died during their stay in the neonatal unit compared with 19% of the treated infants. There was no effect on pulmonary air leaks (pneumothorax and pulmonary interstitial emphysema), and the effects on IVH and chronic lung disease were not seen in this larger trial. Of the infants treated with ALEC, 64% developed HMD compared with 75% of the control infants, and there was a trend towards less severe HMD in the treated infants compared with the controls. Thus, ALEC was associated with improved survival, a slight reduction in the incidence of HMD, and a decrease in the severity of HMD in infants of less than 30 weeks gestation. The effects were not evident for the first six hours and there was no effect on the incidence of pulmonary air leaks. The slower onset of action of ALEC compared with the modified natural surfactants may explain the lack of effect on pulmonary air leaks.

STUDIES COMPARING PROPHYLAXIS AND RESCUE THERAPY

There have been several randomised controlled trials comparing SRT given prophylactically at birth with rescue administration in very low birth weight infants suffering from HMD. Natural human surfactant decreased the incidence of pulmonary interstitial emphysema and improved gas exchange in infants treated with either prophylactic or rescue therapy compared with infants receiving placebo.[208] Prophylactic therapy with human surfactant was, however, associated with an increased incidence of retinopathy of prematurity when compared with rescue therapy.[208] At follow up, significantly more infants in the prophylaxis group suffered from chronic lung disease, defined as a need for supplemental oxygen with radiographic changes at 38 weeks post-conceptional age, compared with infants treated with rescue therapy.[209] The infants who received prophylaxis had significantly lower mental and psychomotor Bayley scores at 12 months of age than infants who received rescue therapy.[209] Thus, there is no advantage to the prophylactic administration of natural human surfactant.

Table 7 Meta-analysis of studies of prophylaxis compared with rescue with surfactant therapy

	Control	*Prophylaxis*	*Rescue*
Incidence of IVH	35.2 (9.6)	35.6 (6.7)	32.9 (6.4)
Pneumothorax	22.8	7.8 (3.7)	7.5 (2.1)
BPD	25.5 (7)	24.6 (6.8)	16.3 (5.5)
Survival	72.9	82.6 (2.4)	82.5 (1.6)
Survival without BPD	51.9	61.9 (4.5)	64 (8.6)

IVH = intraventricular haemorrhage; BPD = bronchopulmonary dysplasia.

Modified natural CLSE was used in a multicentre randomised trial comparing prophylactic administration with rescue therapy in infants of <30 weeks gestation.[210] One minute Apgar scores were significantly lower in the group receiving prophylactic therapy. There was a significant decrease in the incidence of pneumothorax and a significant increase in survival in the infants receiving prophylaxis which was apparent only in infants at 26 weeks gestation. There were no differences in the incidence of chronic lung disease in either group, nor in the incidences of other acute problems such as IVH, PDA, necrotising enterocolitis, and retinopathy of prematurity. One major problem with this study is that the infants who received rescue therapy appeared to have had more severe disease which makes interpretation of the benefits of prophylaxis very difficult. Another randomised trial comparing prophylaxis with rescue therapy with CLSE found no differences in the incidence of air leak, BPD, or survival.[211] Although there were significantly more infants in the prophylactic group who needed oxygen at 28 days gestation than in the rescue group, there were no significant differences in the number of infants requiring oxygen at 36 weeks post-conception, thus suggesting that there was no difference in the incidence of chronic lung disease between the two groups. Apgar scores at one minute were significantly depressed in the group receiving prophylaxis. The authors concluded that there "appears to be no clinical justification for routinely using a prophylactic approach ... in this population of neonates".[211]

Infants of 29–32 weeks gestation receiving prophylactic administration of CLSE had significantly fewer treatment failures as defined by the need for additional dosages and also had lower oxygen requirements than infants receiving rescue therapy.[212] Infants at 30 weeks gestation in the prophylaxis group had a lower mortality rate and decreased need for supplemental oxygen at 28 days. There were no differences in acute complications such as air leaks, retinopathy of prematurity, and intraventricular haemorrhage. Infants receiving prophylactic therapy had significantly lower Apgar scores at one minute and there were significantly more administration errors in this group which included deposition of surfactant into the pharynx.

As shown in table 7 and fig 3, a meta-analysis comparing rescue therapy with prophylaxis shows that there is no advantage of prophylaxis in terms of the occurrence of IVH, survival, and survival without BPD. In fact, the relative risk of BPD is lowered in those receiving rescue

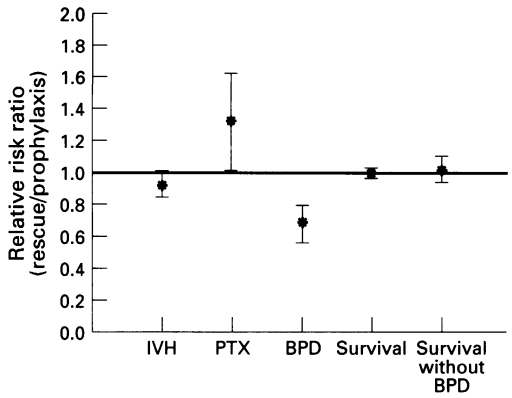

Figure 3 Meta-analysis of prophylaxis versus rescue trials using modified natural surfactant. Data represent the mean ± 95% confidence intervals for relative risk ratios for intraventricular haemorrhage (IVH), pneumothorax (PTX), bronchopulmonary dysplasia (BPD), survival, and survival without BPD. Confidence intervals which do not include the risk ratio of 1 indicate significant reductions (<1) or increases (>1) in the chances for a particular outcome variable.

therapy compared with those who received prophylaxis. There appear to be no differences in the duration of hospital stay between infants receiving prophylactic therapy and those given rescue therapy. As mentioned above, infants receiving prophylactic therapy have lower Apgar scores at one minute[210–212] and one study showed lower Apgar scores at both one and five minutes.[170] This raises the spectre of impacting adversely on long term outcome in infants receiving surfactant therapy prophylactically, especially before their stabilisation in the delivery room.

Given the lack of significant improvement in long term outcome in infants receiving prophylactic therapy, the significant disadvantages of delaying stabilisation in the delivery room, and the administration of surfactant to infants who might not have developed respiratory distress, it appears that prophylaxis offers no advantage over rescue therapy, especially in infants of >26 weeks gestational age. Treatment of infants with established HMD as early as possible appears to be prudent, however, as it lessens mortality, reduces short term complications such as air leaks, and improves survival without chronic lung disease.

SRT is now considered a standard of care for infants suffering from HMD. Administration of SRT must be performed by qualified neonatologists in level III neonatal intensive care units with all of the ancillary services, monitoring capabilities, and expertise in the management of neonates on mechanical ventilatory support. Indeed, the American Academy of Pediatrics has emphasised that SRT, which is now part of the armamentarium of therapeutic interventions in premature infants, should be administered only by neonatal specialists working in level III intensive care nurseries.[213]

Future research will help to answer may important unresolved questions. What is the optimal dose of surfactant? What is the optimal preparation? Is there a gestational age below which prophylaxis is clearly beneficial? What are the effects of surfactant therapy on endogenous pulmonary surfactant production and metabolism? What other respiratory disorder will benefit from such therapy? How is cerebral metabolism and function affected by surfactant therapy?

The intense study of SRT in animals and in humans has become a paradigm for the blending of basic science and clinical science which has advanced the state of medical practice and impacted favourably on the survival and quality of life of our most tiny patients.

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