Clinical review: Exogenous surfactant therapy for acute lung injury/acute respiratory distress syndrome – where do we go from here?

Ahilanandan Dushianthan*1,2, Rebecca Cusack1, Victoria Goss2, Anthony D Postle2 and Mike PW Grocott1,2

Abstract

Acute lung injury and acute respiratory distress syndrome (ARDS) are characterised by severe hypoxemic respiratory failure and poor lung compliance. Despite advances in clinical management, morbidity and mortality remains high. Supportive measures including protective lung ventilation confer a survival advantage in patients with ARDS, but management is otherwise limited by the lack of effective pharmacological therapies. Surfactant dysfunction with quantitative and qualitative abnormalities of both phospholipids and proteins are characteristic of patients with ARDS. Exogenous surfactant replacement in animal models of ARDS and neonatal respiratory distress syndrome shows consistent improvements in gas exchange and survival. However, whilst some adult studies have shown improved oxygenation, no survival benefit has been demonstrated to date. This lack of clinical efficacy may be related to disease heterogeneity (where treatment responders may be obscured by nonresponders), limited understanding of surfactant biology in patients or an absence of therapeutic effect in this population. Crucially, the mechanism of lung injury in neonates is different from that in ARDS: surfactant inhibition by plasma constituents is a typical feature of ARDS, whereas the primary pathology in neonates is the deficiency of surfactant material due to reduced synthesis. Absence of phenotypic characterisation of patients, the lack of an ideal natural surfactant material with adequate surfactant proteins, coupled with uncertainty about optimal timing, dosing and delivery method are some of the limitations of published surfactant replacement clinical trials. Recent advances in stable isotope labelling of surfactant phospholipids coupled with analytical methods using electrospray ionisation mass spectrometry enable highly specific molecular assessment of phospholipid subclasses and synthetic rates that can be utilised for phenotypic characterisation and individualisation of exogenous surfactant replacement therapy. Exploring the clinical benefit of such an approach should be a priority for future ARDS research.

Introduction

Acute respiratory distress syndrome (ARDS), first described by Ashbaugh and colleagues in 1967 [1], is a leading cause of morbidity and mortality in critically ill patients. Outside clinical trial settings, mortality still remains as high as 50% [2]. Diffuse alveolar damage is a typical histopathological feature of ARDS [3]. Three sequential pathological stages of exudation, cellular proliferation and fibrosis are commonly recognised. In the early exudative phase, alveolar epithelial and endothelial injury lead to the accumulation of protein-rich pulmonary oedema. This phase is followed by varying degrees of type II cell proliferation, accumulation of fibroblasts and myofibroblasts associated with collagen deposition in the extracellular matrix, and in some patients this leads to fibrosis [3]. The clinical consequences of the initial injury are refractory hypoxemia and poor lung compliance necessitating mechanical ventilatory support. The diagnostic criteria established by the American–European Consensus Conference in 1994 encompass simple physiological, laboratory and radiological variables [4] but are limited by low specificity and substantial interobserver variability [5,6]. According to these criteria, ARDS is diagnosed by PaO2/FiO2 ratio ≤200 mmHg with bilateral infiltrates on chest radiograph in the absence of raised left atrial hypertension. Acute lung injury (ALI) is defined by the same criteria as ARDS, but with a lesser degree of hypoxemia (PaO2/FiO2 ≤300 mmHg) [4].
ARDS/ALI may result from both direct lung injury (for example, pneumonia, aspiration, drowning and toxic inhalation) and indirect lung injury (for example, sepsis, trauma, blood transfusion and pancreatitis) [3], causing significant phenotypic heterogeneity among patients. Variability in both patients and pathology may explain the disappointing results from many ARDS clinical trials. Indeed, some authors question whether it is reasonable to cohort such varied pathologies within a single unifying diagnostic syndrome [7]. An expert consensus panel has recently proposed a new diagnostic definition (Berlin Definition of ARDS) [8], which subgroups patients according to disease severity defined by the degree of hypoxemia: mild (PaO$_2$/FiO$_2$ ≤300 mmHg); moderate (PaO$_2$/FiO$_2$ ≤200 mmHg); and severe (PaO$_2$/FiO$_2$ ≤100 mmHg). While this definition may facilitate a stratified treatment approach based on severity of hypoxemia, it does not take into account the clinical heterogeneity related to mechanism of injury [8].

Although surfactant alterations are implicated in the pathogenesis of ARDS, surfactant replacement remains of unproven benefit in adult patients. A number of issues relating to human surfactant biology and ARDS may have implications for the design of future surfactant replacement clinical trials and therefore merit closer scrutiny.

**Human surfactant system in health**

The complex mammalian ventilatory system is primarily dependent on surfactant to stabilise alveolar air sacs during respiration. Surfactant is a complex mixture of lipoproteins synthesised, secreted and recycled by type II alveolar cells. Phospholipids make up most of the lipid component of surfactant, but lower levels of neutral lipids such as cholesterol also present. Phosphatidylcholine (PC) is the dominant phospholipid subclass accounting for ~70% of pulmonary surfactant, with phosphatidylglycerol and to a lesser extent phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and sphingomyelin accounting for the rest [9] (Table 1). PC16:0/16:0 (dipalmitoyl phosphatidylcholine (DPPC)) is the most abundant PC molecule and has unique characteristics of alveolar surface tension reduction in humans [10]. Other PC species – notably PC16:0/18:2, PC16:0/16:1, PC16:0/14:0 and PC16:0/18:2 – make up the majority of the rest of surfactant PC composition [11] (Table 2). The relative proportions of these PC species differ among mammalian species, suggesting the possibility of functional variation [12].

Four surfactant proteins have so far been identified (SP-A to SP-D). SP-B and SP-C are hydrophobic proteins, and their primary function is to facilitate the surface tension-reducing characteristics of surfactant phospholipids. SP-A and SP-D are hydrophilic collectin proteins, which are involved in the innate immunity. They have collagen-like domains, which are pattern recognition molecules that facilitate the interaction and removal of a variety of microorganisms and antigens [13].

**Surfactant abnormalities in ARDS**

Earlier postmortem studies have suggested a defective surfactant with reduced surface-lowering characteristics might be contributing to the development of ARDS [1,14]. A number of studies since then have assessed the compositional changes in surfactant from bronchoalveolar lavage fluid (BALF) of patients with ARDS [11, 15-20]. Despite substantial variation in patient characteristics and study methodology, consistent changes in surfactant composition are apparent in these patients (Table 3).

**Total phospholipid concentration**

Determining total phospholipid concentrations in the alveolar surfactant pool is difficult and depends on many factors including the lavage technique, the surface area lavaged and the amount of lavage fluid recovered. These technical issues are reflected in the variability of the results in published clinical studies. An early study by Hallman and colleagues showed normal surfactant phospholipid concentrations in patients with ARDS [15]. However, subsequent studies have shown consistently

| Table 1. Phospholipid composition of the human surfactant system [9] |
|---------------------------------|--------------------------|
| Phospholipid subclass           | Composition (% of total phospholipid) |
| Phosphatidylcholine             | 68                       |
| Phosphatidylglycerol            | 10                       |
| Phosphatidylethanolamine        | 5                        |
| Phosphatidylinositol            | 4                        |
| Phosphatidylserine             | 2                        |
| Sphingomyelin                   | 4                        |

| Table 2. Molecular phosphatidylcholine composition of the human surfactant system [11] |
|---------------------------------|-------------------------|
| PC species                      | Composition (% of total PC) |
| PC16:0/14:0                    | 7.2                     |
| PC16:0/16:1                    | 4.8                     |
| PC16:0/16:0                    | 60.6                    |
| PC16:0/18:2                    | 5.4                     |
| PC16:0/18:1                    | 9.7                     |
| PC16:0/20:4                    | 1.9                     |
| PC18:0/18:2                    | 1.5                     |
| PC18:1/18:1                    | 3.2                     |
| PC18:0/18:1                    | 2.1                     |

PC, phosphatidylcholine.
lower total BALF surfactant phospholipid concentrations in patients with ARDS [16,17], particularly in those where the precipitating cause was pneumonia [17].

**Phospholipid and phosphatidylcholine composition**

The surfactant phospholipid composition in ARDS is characterised by a relative decrease in the fractional concentrations of PC and phosphatidylglycerol with an increase in phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine and sphingomyelin. The assessment of surfactant composition has been limited by the analytical methods available to quantify PC composition, which have lacked precision in identifying molecular species. Two methodological limitations are particularly important. First, quantifying total disaturated PC as a surrogate for DPPC using osmium tetroxide [15,21] has an inherent limitation because this technique also measures other disaturated molecular species such as PC16:0/14:0. Second, assessment of the relative content of individual fatty acids by chromatographic techniques does not reveal the specific molecular structure of individual surfactant PC components [20]. When high-performance liquid chromatography (HPLC) was used to analyse specific molecular PC species in a study of BALF from patients with ARDS, lower concentrations of DPPC and increases in other unsaturated PC species – particularly PC16:0/18:1, PC16:0/18:2, PC18:0/18:2 and PC16:0/20:4 – were demonstrated [11].

**Surfactant protein concentration**

There is an increased total protein concentration in BALF of patients with ARDS [11,18]. This is coupled with a reduction in surfactant-associated proteins SP-A, SP-B and SP-C [11,22]. SP-D concentrations in BALF may remain relatively unchanged during the disease process [22].

**Surfactant aggregates**

Large surfactant aggregates composed of lamellar bodies, tubular myelin and large multilamellar vesicles are highly surface active [23]. During surfactant turnover prior to endocytosis these large aggregates are converted to inactive small aggregates composed of unilamellar vesicles. The exact mechanism leading to this conversion is not fully understood. Reduction in large surfactant aggregates with a relative increase in small aggregates is characteristic of surfactant from patients with ARDS [11,17]. Reduced levels of large surfactant aggregates are also associated with low survival rates [11].

**Possible mechanisms underlying compositional alterations and dysfunctional surfactant in ARDS**

The pathogenesis of surfactant changes in ARDS is poorly understood. Although several animal lung injury models have been utilised, the relevance and applicability of these models in human ARDS remain uncertain. Translation from animal and in vitro models of surfactant dysfunction suggests the possibility of several pathological mechanisms causing surfactant compositional and functional alterations during lung injury. These include reduced surfactant synthesis by injured type II cells, surfactant functional inhibition by plasma constituents, and increased breakdown by activated oxidative and hydrolytic pathways (Table 4). Each of these mechanisms probably contributes to surfactant dysfunction to varying degrees in individual patients.

**Synthetic dysfunction**

In ARDS, synthetic or secretory dysfunction may result from direct or indirect injury to alveolar type II cells. Animal models of hyperoxia-induced direct lung injury show decreased surfactant PC synthesis [24]. In contrast, subcutaneous injection of nitrogenated urethane compound (N-nitroso-N-methyl-urethane), which is toxic to type II cells, leads to increased surfactant saturated PC synthesis and secretion [25]. This paradoxical finding highlights the limitations of animal lung injury models and may be due to the different mechanisms of injury in these studies.

Stable isotope labelling of surfactant precursors is a novel approach to study surfactant kinetics in humans [26]. One in vivo study using such a method suggests

### Table 3. Surfactant abnormalities seen in clinical studies of ARDS/ALI patients

| Surfactant characteristic | Abnormalities in ALI/ARDS |
|--------------------------|--------------------------|
| Surface activity         | Reduced surface tension  |
| Phospholipid profile     | Reduced levels and fractional concentrations of phosphatidylcholine and phosphatidylglycerol with increase in fractional concentrations of phosphatidylinositol, phosphatidylethanolamine, phosphatidylserine and sphingomyelin |
| Phosphatidylcholine composition | Reduced levels and fractional concentrations of dipalmityl phosphatidylcholine with increased fractional concentration of unsaturated species |
| Surfactant aggregates    | Reduced proportion of large aggregates to small aggregates |
| Surfactant proteins      | Decreased alveolar surfactant proteins and increased plasma surfactant proteins |

ALI, acute lung injury; ARDS, acute respiratory distress syndrome.
Table 4. Possible reasons for surfactant abnormalities in acute respiratory distress syndrome/acute lung injury

Reduced surfactant synthesis and recycling by injured type II cells
Increased breakdown by hydrolysis and proteolysis
Oxidative injury by reactive oxygen species
Dilution of surfactant material by florid oedema/fluid
Dysfunctional surfactant film formation due to accumulation of plasma constituents
Inhibition by competitive adsorption of plasma proteins

there is an increased surfactant PC synthesis in ARDS patients compared with ventilated controls [27]. In contrast to this, sequential quantitative BALF studies in ARDS patients have shown consistently lower concentrations of PC [18] and DPPC [11]. Surfactant SP-D is primarily secreted by type II cells and can be used as a surrogate biomarker for alveolar epithelial injury [28]. Although BALF SP-D levels remain relatively unchanged during the disease course, lower SP-D levels are evident in a subgroup of ARDS patients and are associated with a significant increase in mortality [22]. Variation in the volume of BALF recovery may in part explain these findings. Another possible explanation is variable surfactant synthetic and secretory patterns amongst patients with similar clinical pictures: in other words, phenotypic variation that can only be identified through in vivo characterisation of surfactant metabolism.

**Surfactant functional inhibition**
During the early stages of ARDS, there is flooding of plasma material (plasma proteins, red blood cells, fibrin and fibrin degradation products) into the alveolar space due to endothelial and epithelial injury. In experimental models, plasma proteins – in particular, albumin, haemoglobin, fibrinogen and fibrin monomers have been shown to impair surfactant function and hence increase surface tension [29]. Reconstitution of protein extracted from BALF supernatant resulted in further deterioration of surface tension, suggesting an inhibitory effect of leaked plasma proteins within the alveolar space [17]. Although the exact mechanisms of surfactant functional inhibition by plasma constituents are not well established, competitive adsorption of plasma proteins [29] and dysfunctional surfactant film formation [30] have both been postulated.

**Increased surfactant breakdown by oxidation**
Reactive oxygen species are generated as a part of normal oxygen metabolism and are physiologically active in cell signalling. Various reactive oxygen species, including hydrogen peroxide, superoxide and nitric oxide, are released by enzymatic reactions (xanthine oxidase/glucose oxidase/nicotinamide adenine dinucleotide phosphate oxidase) from inflammatory cells. Oxidative disruption of lipids and proteins leads to dysfunctional surfactant and this has been postulated as contributory to the pathogenesis of lung injury [31]. Exogenous surfactant is also subject to reactive oxygen species mediated oxidation, leading to diminished surface tension-reducing characteristics [32].

**Increased surfactant breakdown by hydrolysis**
Secretory phospholipase A₁ (PLA₁) activity is increased in BALF from patients with ARDS [33]. PLA₁-mediated hydrolysis of surfactant phospholipids contributes directly to surfactant dysfunction and generates free fatty acids that further inhibit surfactant function [34,35]. Hydrolysis of surfactant PC leads to lysophosphatidylcholine formation, and higher levels of PLA₁ activity and lysophosphatidylcholine concentration in ARDS are associated with increased mortality [33,36].

**Surfactant replacement in ARDS/ALI**
Fujiwara and colleagues reported the first positive, uncontrolled, clinical study of surfactant replacement in 1980 [37]. In this study, 10 premature neonates with severe respiratory distress syndrome were successfully treated with natural bovine surfactant supplemented with synthetic DPPC and phosphatidylglycerol. Subsequent randomised clinical trials (RCTs) with both natural and synthetic surfactant preparations have shown consistent improvements in lung mechanics, oxygenation and mortality in neonatal respiratory distress syndrome [38]. Several RCTs of surfactant replacement in adults with ARDS have been conducted since 1994 [39-46]. These generally have shown improvements in oxygenation indices but have failed to produce any demonstrable survival benefits [47].

An initial phase I study of synthetic surfactant composed of DPPC without any surfactant proteins (Exosurf) demonstrated its safety profile in 51 patients with sepsis-induced ARDS [39]. In this study, surfactant was nebulised for 5 days continuously and a trend towards mortality benefit was seen in the treatment group. A subsequent larger RCT using the same methods with the same surfactant preparation and study population failed to show any benefits in oxygenation, mortality, length of ICU stay or duration of mechanical ventilation [40]. These negative results may be explained by the lack of surfactant proteins in the surfactant preparation leading to reduced surface spreading characteristics and poor alveolar surfactant deposition by this delivery method (estimated only 5% deposition) [40].

Gregory and colleagues performed a phase II RCT using bovine lung extract containing phospholipids, neutral lipids, fatty acids and surfactant proteins SP-B and SP-C in patients with ARDS. This study enrolled 59
patients and demonstrated improved oxygenation and a trend towards reduced mortality in the surfactant group [41].

A European-based multicentre RCT using a large bolus tracheal instillation of freeze-dried natural porcine surfactant (HL-10) failed to show any mortality benefit. This surfactant preparation consisting of phospholipids (90 to 95%) and SP-B and SP-C (1 to 2%) was instilled for up to three doses (totalling 600 mg/kg). This study of 418 ARDS/ALI patients was terminated early due to an excess of serious adverse events, such as hypotension and hypoxemia, in the treatment group [42].

Spragg and colleagues conducted three RCTs with a surfactant preparation consisting of phospholipids and recombinant SP-C. Following positive results from an animal study [48], a phase II study of recombinant SP-C in 40 ARDS patients showed a good safety profile [43]. This study was followed by a large multicentre phase III RCT of 448 ARDS patients, which showed improved oxygenation but no overall mortality benefit in the treatment group [44]. However, post hoc analysis demonstrated a trend towards mortality benefit for those patients with direct lung injury from aspiration and pneumonia [44]. Following this study, a further phase III RCT was conducted in a large cohort of patients (844 patients) with severe hypoxemia secondary to aspiration and pneumonia. There was no mortality benefit and the study was terminated early due to futility. Furthermore, contrary to the results of previous studies, this study reported a lack of improvement in oxygenation and increased treatment-related serious adverse events [46]. In this study, however, the surfactant preparation process involved a shearing step where it was passed forcefully through a narrow channel to improve suspension and distribution. This shearing step may have resulted in reduced surface tension-lowering properties by possibly exposing the exogenous surfactant to functional inhibition by plasma proteins [46].

Improved gas exchange was noted in nonrandomised clinical studies with bronchoscopic administration of natural porcine surfactant [49] and bovine surfactant [50,51]. However, these studies were small, noncontrolled and their findings have not been replicated in larger randomised controlled studies.

Possible explanations for the negative results from surfactant replacement studies in ARDS

No large RCT of exogenous surfactant replacement has shown reduced mortality from this intervention (Table 5). This finding has been confirmed by a recent systematic review and meta-analysis that included nine RCTs with a total of 2,575 patients, which found no evidence of a mortality benefit. However, the validity of this result is limited by the substantial clinical heterogeneity of the studies included in this analysis [47]. Possible reasons for this failure of a theoretically promising therapy include the differences in the exogenous surfactant composition, drug delivery methods and the presence of variation in surfactant biology among the target population.

Exogenous surfactant composition

Surfactant preparations vary according to their composition, biophysical activity, susceptibility to functional inhibition, preparation technique and associated costs. The relative composition of DPPC and surfactant proteins influences surface tension-lowering characteristics of the exogenous surfactant. Although DPPC is the primary surface tension-lowering molecule, pure DPPC preparations are limited by their lack of surface spreading and adsorption characteristics [39,40]. Although recent studies with recombinant SP-C-based surfactant preparation showed no mortality benefit [44,46], manipulation of the composition by adding other surfactant proteins may potentiate its clinical effect. For instance, SP-A has been shown to improve phospholipid adsorption and surface activity and may reduce conversion of large to small aggregates [52,53]. Developing surfactant preparations that more closely reflect natural human surfactant in the alveolus may therefore improve clinical outcome. In the neonatal population, natural surfactants from lavage or homogenised lung are clinically more effective than synthetic preparations [54]. However, compared to neonates, a large amount of exogenous surfactant is needed to provide adequate treatment in adults. Furthermore, replacement strategies using natural surfactant preparations are costly due to the laborious extraction techniques.

Surfactant delivery methods

Clinical studies with nebulised surfactant preparations conducted in the 1990s were limited by poor alveolar deposition [39,40]. Much of the published work since then has evaluated surfactant delivery via intratracheal instillation [41-46]. A large quantity of surfactant material is generally delivered by this route, which may be of benefit in counteracting the effect of surfactant inhibition [42]. However, this large quantity delivery can result in flooding of the central airways and lead to increased airway resistance and worsening of hypoxemia. In animal models, intratracheal administration leads to poor surfactant deposition in the collapsed alveoli [55]. An alternative is sequential bronchoscopic administration, which has been evaluated in a number of uncontrolled studies. Bronchoscopic sequential segmental administration of natural bovine surfactant was associated with improved PaO₂/FiO₂ ratios coupled with improved ventilation–perfusion matching in the lungs [50], as well
Table 5. Characteristics of surfactant replacement RCTs in ARDS/ALI

| Study                        | Design   | Cohort                      | Number of patients | Surfactant type         | Delivery mode and dose | Outcome                                                                                           | Comments                                                                                      |
|------------------------------|----------|-----------------------------|--------------------|-------------------------|------------------------|---------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
| Spragg and colleagues [44]   | Multicentre RCT, phase III | Direct lung injury with PaO₂/FIO₂ ≤170 mmHg (aspiration+ pneumonia) | 843                | rSP-C based (synthetic), 1 ml = 1 mg rSP-C and 50 mg PLs | Intratracheal, 1 ml/kg LBW for maximum of eight doses until 96 hours | 1. No difference in 28-day mortality, oxygenation or ventilator free days 2. Similar adverse events | 1. Differ from other rSP-C studies by no improvement in oxygenation 2. Shearing step used to improve dispersion may have altered the property 3. Prematurely stopped due to futility |
| Kesecioglu and colleagues [42]| Multicentre RCT, phase III | ALI/ARDS                  | 418                | HL10 – freeze dried natural porcine surfactant (90-95% phospholipids and 1-2% of SP-B and SP-C) | Intratracheal, up to three doses – cumulative doses of 600 mg/kg at 0, 12, 36 hours | 1. Increased trend towards mortality in surfactant group with no improvements in secondary outcomes such as oxygenation and SOFA scores 2. More adverse events in the surfactant group | 1. Prematurely terminated due to futility |
| Markart and colleagues [45]  | Multicentre RCT, phase I/II | ARDS                        | 31                 | rSP-C based (synthetic), 1 ml = 1 mg rSP-C and 50 mg PLs | Intratracheal, 1 ml/kg LBW up to four doses in the first 24 hours | 1. Improved gas exchange in surfactant group 2. Normalisation of surfactant PLs and proteins | 1. Not designed to assess mortality 2. Treatment period was 24 hours |
| Spragg and colleagues [44]   | Multicentre RCT, phase III | ARDS                        | 221 and 227       | rSP-C based (synthetic), 1 ml = 1 mg rSP-C and 50 mg PLs | Intratracheal, 1 ml/kg LBW up to four doses at 4-hour intervals in the first 24 hours | 1. No difference in survival or ventilator free days but improved oxygenation in the surfactant group 2. More adverse events in the surfactant group in the first 24 hours after treatment | 1. Post hoc analysis for intrinsic ARDS showed trend towards improved mortality. 2. Treatment period was 24 hours |
| Spragg and colleagues [43]   | Multicentre RCT, phase I/II | ARDS                        | 40                 | rSP-C based (synthetic), 1 ml = 1 mg rSP-C and 50 mg PLs | Intratracheal, two groups: group 1, 1 ml/kg LBW; group 2, 0.5 mg/kg LBW, up to four times in the first 24 hours | 1. Safety was comparable with no differences in oxygenation and ventilator free days 2. Decreased plasma IL-6 in group 1 | 1. Treatment period was 24 hours |
| Gregory and colleagues [41]  | Multicentre RCT, phase II/III | ARDS                        | 59                 | Natural bovine lung extract (Survanta; contains phospholipids, neutral lipids, fatty acids, and surfactant proteins with additional DPPC, palmitic acid and tripalmitin) | Intratracheal, three groups: group 1, 8×50 mg/kg LBW; group 2, 4×100 mg/kg LBW; group 3, 8×100 mg/kg LBW | 1. Oxygenation was better with surfactant group 2 2. Trend towards improved mortality in groups 2 and 3 | 1. Small number of patients in each group |
| Anzueto and colleagues [40]  | Multicentre RCT, phase III | Sepsis-induced ARDS        | 725                | Exosurf (synthetic), 13.5 mg DPPC/ml | Aerosol, 112 mg DPPC/kg/day for 5 days | 1. No difference in 30 day mortality, oxygenation or mean number of ventilation days | 1. Only sepsis cohort was studied 2. Aerosolised preparation with poor alveolar deposition 3. No surfactant proteins in the preparation |
| Weg and colleagues [39]      | Multicentre RCT, phase II | Sepsis-induced ARDS        | 51                 | Exosurf (synthetic), 13.5 mg DPPC/ml | Aerosol, two groups: group 1, 21.9 mg DPPC/kg/day; group 2, 43.5 mg DPPC/kg/day. Aerosolised for either 12 or 24 hours for 5 days | 1. Safety was comparable between three groups | 1. Aerosolised preparation with poor alveolar deposition 2. No surfactant proteins in the preparation |

ALI, acute lung injury; ARDS, acute respiratory distress syndrome; DPPC, dipalmitoyl phosphatidylcholine; IL, interleukin; LBW, lean body weight; PL, phospholipid; RCT, randomised controlled trial; rSP-C, recombinant surfactant protein C; SOFA, Sequential Organ Failure Assessment score.
as relative normalisation of surfactant composition and function [51]. However, this technique is time consuming and resource intensive and may not be feasible in clinical practice or large RCTs.

Uniform delivery of surfactant material to both affected and unaffected parts of the lung might improve atelectasis in affected areas, but may be detrimental in unaffected areas. Computerised tomography has demonstrated the heterogeneous distribution of lung injury in ARDS, with the lower zones of the lungs tending to be most affected [56]. Computerised tomography images may help to guide targeted bronchoscopic administration of exogenous surfactant to affected lobes.

**Clinical heterogeneity of surfactant biology in ARDS**

ARDS encompasses a variety of aetiologies leading to an apparently common diffuse alveolar damage. Consequently, surfactant alterations are attributed to several pathological mechanisms, which have not been fully explored by human studies. Specifically, patterns of surfactant synthesis and metabolism have not been characterised in ARDS. Current ARDS diagnostic definitions are uninformative with regards to the degree of alveolar injury, the dynamic surfactant pool and surfactant metabolism. Phenotypic characterisation of groups with surfactant synthetic dysfunction may help to target those patients most likely to benefit from exogenous surfactant replacement. For example, reduced surfactant synthesis in neonatal respiratory distress syndrome provides the best human surfactant-deficient lung injury model – and in this context, exogenous surfactant replacement leads to reduced morbidity and mortality [38].

Where there is intact alveolar synthetic function, assessment of the degree of surfactant inhibition or breakdown by hydrolysis and oxidation may be of value. Testing of patient’s endogenous surfactant against the exogenous surfactant material may provide important clues to the degree of functional inhibition that may be encountered during supplementation. Attempts to counteract this by instilling large amounts of exogenous surfactant may result in an increased risk of serious adverse events [42]. Addition of other surfactant proteins such as SP-A or SP-B may counteract functional inhibition by plasma constituents and has been shown to improve the efficacy of exogenous surfactant preparations in *in vitro* studies [57,58].

The combination of increased PLA₂ activity in BALF and increased concentrations of lysophospholipids may serve as markers of phospholipase-mediated surfactant phospholipid breakdown. This breakdown could be counteracted by PLA₂-resistant surfactant analogues such as phospholipase-resistant diether lipids [59], PLA₂ inhibitors or SP-A [60].

Exogenous surfactant subjected to oxidation has reduced surface activity [61,62] and this may have contributed to the negative clinical outcomes in ARDS trials. Oxidative metabolites of phospholipids can be quantified by mass spectrometry [63]. These oxidised phospholipids may be used as a surrogate to assess the degree of oxidation mediated surfactant breakdown. The potential of oxidised phospholipids as biomarkers in this context has not been fully explored. SP-A and SP-D have antioxidant activity [64] and augmentation of these proteins or other endogenous antioxidants such as superoxide dismutase, vitamin E, melatonin and ebselen may moderate oxidative breakdown of exogenous surfactant in those with high levels of surfactant oxidation [65].

**Can we phenotype patients according to surfactant synthetic function?**

Clinical trials of exogenous surfactant have not so far phenotyped patients according to alveolar surfactant synthesis, metabolism and degree of functional inhibition prior to replacement strategies. Improved characterisation of synthetic ability and surfactant function may identify selected patient groups that may benefit from specific surfactant therapies individualised according to the underlying pathophysiological processes.

In patients with ARDS, dynamic surfactant assessment involves consecutive BALF analyses to demonstrate time-dependent changes in surfactant composition [11,22]. These studies have inherent limitations, including variable BALF recovery and lack of information regarding synthesis or turnover. Radio-isotope-labelling studies conducted in previous decades provided substantial knowledge regarding the nature of surfactant dynamics in animal models of lung injury, but these techniques are...
Surfactant PC is synthesised from phospholipid precursors such as glucose, glycerol and choline via the cytidine diphosphate–choline pathway (Figure 1). By labelling these phospholipid precursors with stable (non radioactive) isotopes, it is possible to assess surfactant PC synthetic rates and metabolism. 13C-labelled glucose and free fatty acids, such as labelled 13C-palmitic acid (16:0), have been used successfully to study surfactant metabolism in neonates [66]. The fractional synthetic rates (percentage of newly synthesised surfactant per day) of disaturated surfactant PC (Palmitate 16:0) can be quantified using gas chromatography–isotope ratio mass spectrometry (GC-IRMS) by the detection of incorporated labelled 13C. However, fatty acid labelling only provides information regarding metabolism of that particular fatty acid in question and the assessment of 13C enrichment using GC-IRMS is not informative for the synthesis and metabolism of other individual surfactant PC species [26].

An alternative technique is the use of deuterium, which is a naturally occurring stable isotope of hydrogen. Isotope labelling of choline with nine deuterium atoms, which increases the number of mass units by +9 in the PC head group in subsequent metabolic products, helps to trace specific PC molecular species in pulmonary surfactant. Advances in analytical techniques with the evolution of electrospray ionisation mass spectrometry now allow identification of each species of surfactant PC with high sensitivity using specified scans. Figure 2 shows typical mass spectra for native surfactant PC composition (Figure 2a) and the newly produced deuteriated PC species (Figure 2b). Using this methodology, deuteriated choline incorporation into sputum PC has been assessed in healthy volunteers, demonstrating the feasibility of measuring synthetic rates of individual surfactant PC species.
species [67]. Further studies are needed to evaluate this technique in the alveolar surfactant pool in health and disease states such as ARDS.

**Conclusion**

Knowledge of surfactant biology has evolved over the last 50 years, providing valuable insight into alveolar surfactant physiology in lung injury. However, there remain substantial knowledge gaps that need to be addressed by future research. An ideal surfactant material that mimics the properties of human surfactant is lacking and research should focus on refining surfactant preparations that incorporate all surfactant proteins as well as developing measures to reduce the impact of functional inhibition. Targeting of surfactant delivery to the lobes that are most affected may also be of benefit. Finally, and most crucially, the target population needs to be characterised according to surfactant synthetic function using the best available technology, including nonradioisotope labelling of surfactant precursors. This characterisation may permit stratification of the ALI/ARDS population according to the surfactant synthetic capability of alveolar type II cells and provide a rational basis for targeting exogenous surfactant interventions.

**Abbreviations**

ALI, acute lung injury; ARDS, acute respiratory distress syndrome; BALF, bronchoalveolar lavage fluid; DPPC, dipalmitoyl phosphatidylcholine; FiO₂, proportion of oxygen in the inhaled air; HPLC, high-performance liquid chromatography; PaO₂, partial pressure of arterial oxygen; PC, phosphatidylcholine; PLA₂, phospholipase A₂; RCT, randomised controlled trial; SP, surfactant protein.

**Competing interests**

ADP has no direct financial interest in the work presented in this review; his surfactant research programme is supported by kind donation of a therapeutic surfactant from Chiesi for a clinical trial. The remaining authors declare that they have no competing interests.

**Acknowledgements**

All authors are funded in part by the University Hospitals Southampton NHS Foundation Trust – University of Southampton Respiratory Biomedical Research Unit which received a portion of its funding from the United Kingdom Department of Health’s National Institute of Health Research Biomedical Research Unit funding scheme.

**Author details**

1Anaesthesia and Critical Care Research Unit, CE 93, MP24, E-Level, Centre Block, University Hospital Southampton NHS Foundation Trust, Southampton SO16 6YD, UK. 2Integrative Physiology and Critical Illness, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, University Hospital Southampton NHS Foundation Trust, Southampton SO16 6YD, UK.

**Published: 22 November 2012**

**References**

1. Ashbaugh DG, Bigelow DB, Petty TL, Levine BE. Acute respiratory distress in adults. Lancet 1967, 2:319-323.
2. Villar J, Blanco J, Añón JM, Santos-Bouza A, Bianch L, Ambros A, Gandia F, Carrero D, Mostero F, Basaldúa S, Fernández RL, Kacmarek RM. ALIEN Network: The ALIEN study: incidence and outcome of acute respiratory distress syndrome in the era of lung protective ventilation. *Intensive Care Med* 2011, 37:1932-1941.
3. Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med* 2000, 342:1334-1349.
4. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, Legall JR, Morris A, Spragg R. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med* 1994, 149:818-824.
5. Ferguson ND, Frutos-Vivar F, Esteban A, Fernandez-Segoviano P, Armamburu JA, Najera L, Stewart TE. Acute respiratory distress syndrome: underrecognition by clinicians and diagnostic accuracy of three clinical definitions. *Crit Care Med* 2005, 33:2228-2234.
6. Rubenfeld GD, Caldwell E, Granton J, Hudson LD, Matthay MA. Interobserver variability in applying a radiographic definition for ARDS. *Crit Care Med* 1999, 116:347-1353.

25. Soni N. ARDS, acronyms and the Pinocchio effect. *Anaesthesia* 2010, 65:976-979.
26. Ranieri V, Rubenfeld GD, Thompson B, Ferguson ND, Caldwell E, Fan E, Campopiano L, Slutsky AS, Antonelli M, Anzueto A, Beale R, Brochard L, Brower R, Esteban A, Gattinoni L, Rhodes A, Vincent JL, Bersten A, Needham D. Preseve A. Acute respiratory distress syndrome: the Berlin Definition. *JAMA* 2012, 307:2526-2533.
27. Rooney SA, Young SL, Mendelson CR. Molecular and cellular processing of lung surfactant. *FASEB J* 1994, 8:957-967.
28. Lang CJ, Postle AD, Ongeg S, Possmayer F, Bernard H, Panda AK, Jurgens KD, Milsum WK, Nag K, Daniels CB. Dipalmitoylphosphatidylcholine is not the major surfactant phospholipid species in all mammals. *Am J Physiol Regul Integr Comp Physiol* 2005, 289:R1426-R1439.
29. Schmidt R, Markart P, Ruppert C, Wygocka M, Kuchenbuch T, Wahlmuth D, Seeger W, Guenther A. Time-dependent changes in pulmonary surfactant function and composition in acute respiratory distress syndrome due to pneumonia or aspiction. *Respir Res* 2007, 8:55.
30. Postle AD, Heeley EL, Wilton DC. A comparison of the molecular species compositions of mammalian lung surfactant phospholipids. *Comp Biochem Physiol A Mol Integr Physiol* 2001, 129:655-73.
31. Haagensen MP, Diemer RV. Surfactant-associated proteins: functions and structural variation. *Comp Biochem Physiol A Mol Integr Physiol* 2001, 129:991-108.
32. Petty TL, Silvers GW, Paul GW, Sanford RE. Abnormalities in lung elastic properties and surfactant function in acute respiratory distress syndrome. *Chest* 1979, 75:571-574.
33. Hallman M, Spragg R, Harrell JH, Moser KM, Gluck L. Evidence of lung surfactant abnormality in respiratory failure. Study of bronchoalveolar lavage phospholipids, surface activity, phospholipase activity, and plasma myoinositol. *Clin Invest* 1982, 68:673-683.
34. Gregory TJ, Longmore WI, Maxwel MA, Whitsett JA, Reed CR, Fowler AA, 3rd, Hudson LD, Mauder R, Crim C, Hyers TM. Surfactant chemical composition and biophysical activity in acute respiratory distress syndrome. *J Clin Invest* 1991, 88:1976-1981.
35. Gunther A, Siebert C, Schmidt R, Ziegler S, Grimminger F, Yabut M, Temmesfeld B, Wahlmuth D, Morh H, Seeger W. Surfactant alterations in severe pneumonia, acute respiratory distress syndrome, and cardiogenic lung edema. *Am J Respir Crit Care Med* 1996, 153:176-184.
36. Nakos G, Kitisoulis EI, Tsangaris I, Lekka ME. Bronchoalveolar lavage fluid characteristics of early intermediate and late phases of ARDS. Alterations in leukocytes, proteins, PAF and surfactant components. *Intensive Care Med* 1998, 24:926-933.
37. Pison U, Seeger W, Buchhorn R, Joka T, Brand M, Obertacke U, Neuhoef H, Schmitz-Neuering FP. Surfactant abnormalities in patients with respiratory failure after multiple trauma. *Am Rev Respir Dis* 1989, 140:1033-1039.
38. Schmidt R, Meier U, Yabut-Perez M, Wahlmuth D, Grimminger F, Seeger W, Buchhorn R, Joka T, Brand M, Obertacke U, Neuhoef H, Schmitz-Neuering FP. Surfactant abnormalities in patients with respiratory failure after multiple trauma. *Am Rev Respir Dis* 1989, 140:1033-1039.
to small surfactant aggregates. Biochem J 1993, 295:141-147.
24. Holm BA, Matalon S, Finkelestein JH, Notter RH. Type II pneumocyte changes during hyperoxic lung injury and recovery. J Appl Physiol 1988, 65:2672-2678.
25. Lewis JF, Ikegami M, Jobe AH. Altered surfactant function and metabolism in rabbits with acute lung injury. J Appl Physiol 1990, 69:2833-2830.
26. Postle AD, Hunt AN. Dynamic lipidomics with stable isotope labelling. J Chromatogr A Analyt Technol Biomed Life Sci 2009, 877:2716-2721.
27. Simonato M, Bantussio A, Ori C, Vedovelli L, Rossi S, Dalla ML, Rizzi S, Camielli VP, Cogo PE. Disaturated-phosphatidylcholine and surfactant protein-B turnover in human acute lung injury and in control patients. Respir Res 2011, 12:36.
28. Ware LB, Koyama T, Billheimer DD, Wu W, Bernard GR, Thompson BT, Brower VG. Dynamic lipidomics with stable isotope labelling. J Chromatogr B Analyt Technol Biomed Life Sci 2009, 877:2716-2721.
29. Holm BA, Wang Z, Notter RH. Multiple mechanisms of lung surfactant inhibition. Pediatr Res 1999, 46:655-93.
30. Gunasekara L, Schoel WM, Schurch S, Arminiev M. A comparative study of mechanisms of surfactant inhibition. Biochim Biophys Acta 2008, 1778:833-444.
31. Haagsman HP. Oxidative damage of the pulmonary surfactant system. Semin Neonatol 1998, 3:207-217.
32. Rodriguez-Capote K, Manzanares D, Haines T, Possmayer F. Reactive oxygen species inhibition of surfactant involves structural and functional alterations to surfactant proteins SP-B and SP-C. Biochim Biophys Acta 2006, 90:2808-2821.
33. Kim DK, Fukuda T, Thompson BT, Cockrill B, Hales C, Bonventre JV. In vivo conversion of surfactant subtypes is altered in alveolar surfactant isolated from injured lungs. Am J Respir Crit Care Med 1992, 145:1416-1420.
34. Halliday HL. Oxidative modification and depletion of pulmonary surfactant. Chest 1990, 98:103-118.
35. Dushianthan B. Dynamic lipidomics with stable isotope labelling. J Chromatogr B Analyt Technol Biomed Life Sci 2009, 877:2716-2721.
36. Spragg RG, Taut FL, Lewis JF, Schenk P, Ruppert C, Dean N, Krell K, Karabinis A, Gunther A. Reombinant surfactant protein-C based surfactant for patients with severe direct lung injury. Am J Respir Crit Care Med 2011, 183:1055-1061.
37. Meng H, Sun Y, Lu J, Fu S, Meng Z, Scott M, Li Q. Exogenous surfactant may improve oxygenation but not mortality in adult patients with acute lung injury. J Crit Care 2009, 24:12-7.
38. Lewis J, McCaig L, Hafner D, Spragg R, Veldhuisen R, Kerr C. Dosing and delivery of a recombinant surfactant in lung-injured adult sheep. Am J Respir Crit Care Med 1999, 159:741-747.
39. Spragg RG, Gilliard N, Richman P, Scott M, Huf JD, Pappert D, Robertson B, Cunsted T, Strayer D. Acute effects of a single dose of porcine surfactant on patients with the adult respiratory distress syndrome. Chest 1994, 105:195-202.
40. Walmrath D, Strohm T, Kreit S, Schmehl T, Ruppert C, Vartemberg BB, Béréziat G, Voelker DR, Touqui L. Bronchoalveolar lavage fluid phospholipase A2 activity in human adult respiratory distress syndrome. Am J Respir Crit Care Med 1997, 155:1085-1090.
41. Gregoire TJ, Steinberg KP, Spragg R, Moxley E, Hafner D, Baughman RP. Pulmonary surfactant-associated protein-A and phospholipase A2 protein interaction. J Clin Invest 1998, 102:1152-1160.
42. Seeger W, Gunther A, Thede C. Differential sensitivity to fibrinogen inhibition of SP-C vs. SP-B based surfactants. Am J Physiol Lung Cell Mol Physiol 2010, 298:L380-386.
43. Diemel RV, Walch M, Haagsman HP. The acute respiratory distress syndrome. Swiss Med Wkly 2005, 135:169-174.
44. Siipola R, Lewis J, Ikegami M. In vitro conversion of surfactant subtypes is altered in alveolar surfactant isolated from injured lungs. Am J Respir Dis 2012, 145:1416-1420.
45. Soll RF. Natural surfactant extract versus synthetic surfactant for neonatal respiratory distress syndrome. Cochrane Database Syst Rev 2000, 2:CD000148.
46. Diemel RV, Walch M, Haagsman HP. In vitro and in vivo intrapulmonary distribution of fluorescently labeled surfactant. Crit Care Med 2002, 30:1083-1090.
47. Gattinoni L, Chiurnello D, Cressoni M, Valenza F. Pulmonary computed tomography and adult respiratory distress syndrome. Swiss Med Wkly 2005, 135:169-174.
48. Cockshutt AM, Weitz J, Possmayer F. Pulmonary surfactant-associated protein A enhances the surface activity of lipid extract surfactant and reverses inhibition by blood proteins in vitro. Biochem J 1990, 264:842-849.
49. Seeger W, Gunther A, Thede C. Differential sensitivity to fibrinogen inhibition of SP-C vs. SP-B based surfactants. Am J Physiol Lung Cell Mol Physiol 2010, 298:L380-386.
50. Spragg RG, Gilliard N, Richman P, Scott M, Huf JD, Pappert D, Robertson B, Cunsted T, Strayer D. Acute effects of a single dose of porcine surfactant on patients with the adult respiratory distress syndrome. Chest 1994, 105:195-202.
51. Walmrath D, Strohm T, Kreit S, Schmehl T, Ruppert C, Vartemberg BB, Béréziat G, Voelker DR, Touqui L. Bronchoalveolar lavage fluid phospholipase A2 activity in human adult respiratory distress syndrome. Am J Respir Crit Care Med 1997, 155:1085-1090.
52. Gregoire TJ, Steinberg KP, Spragg R, Moxley E, Hafner D, Baughman RP. Pulmonary surfactant-associated protein-A and phospholipase A2 protein interaction. J Clin Invest 1998, 102:1152-1160.
53. Seeger W, Lepper H, Wolf C, Touqui L. Alveolar surfactant function after exposure to oxidative stress and to oxygenated and native phospholipase A2 protein interaction. J Clin Invest 1998, 102:1152-1160.
54. Seeger W, Gunther A, Thede C. Differential sensitivity to fibrinogen inhibition of SP-C vs. SP-B based surfactants. Am J Physiol Lung Cell Mol Physiol 2010, 298:L380-386.
55. Diemel RV, Walch M, Haagsman HP. In vitro and in vivo intrapulmonary distribution of fluorescently labeled surfactant. Crit Care Med 2002, 30:1083-1090.
56. Gattinoni L, Chiurnello D, Cressoni M, Valenza F. Pulmonary computed tomography and adult respiratory distress syndrome. Swiss Med Wkly 2005, 135:169-174.
57. Cockshutt AM, Weitz J, Possmayer F. Pulmonary surfactant-associated protein A enhances the surface activity of lipid extract surfactant and reverses inhibition by blood proteins in vitro. Biochem J 1990, 264:842-849.
58. Seeger W, Gunther A, Thede C. Differential sensitivity to fibrinogen inhibition of SP-C vs. SP-B based surfactants. Am J Physiol Lung Cell Mol Physiol 2010, 298:L380-386.
65. Bouhafs RK, Jarstrand C: Effects of antioxidants on surfactant peroxidation by stimulated human polymorphonuclear leukocytes. *Free Radic Res* 2002, 36:727-734.

66. Caroni VP, Zimmermann L, Hanwas A, Cogo PE: Pulmonary surfactant kinetics of the newborn infant: novel insights from studies with stable isotopes. *J Perinatol* 2009, 29(Suppl 2):S29-S37.

67. Bernhard W, Pynn CJ, Jaworski A, Rau GA, Hohlfeld JM, Frehorst J, Poets CF, Stoll D, Postle AD: Mass spectrometric analysis of surfactant metabolism in human volunteers using deuteriated choline. *Am J Respir Crit Care Med* 2004, 170:54-58.

Cite this article as: Dushianthan A, et al: Clinical review: Exogenous surfactant therapy for acute lung injury/acute respiratory distress syndrome – where do we go from here? *Critical Care* 2012, 16:238.