Synthetic surfactant containing SP-B and SP-C mimics is superior to single-peptide formulations in rabbits with chemical acute lung injury

Background. Chemical spills are on the rise and inhalation of toxic chemicals may induce chemical acute lung injury (ALI)/acute respiratory distress syndrome (ARDS). Although the pathophysiology of ALI/ARDS is well understood, the absence of specific antidotes besides has limited the effectiveness of therapeutic interventions. Objectives. Surfactant inactivation and formation of free radicals are important pathways in (chemical) ALI. We tested the potential of lipid mixtures with advanced surfactant protein B and C (SP-B and C) mimics to improve oxygenation and lung compliance in rabbits with lavage- and chemical-induced ALI/ARDS. Methods. Ventilated young adult rabbits underwent repeated saline lung lavages or underwent intratracheal instillation of hydrochloric acid to induce ALI/ARDS. After establishment of respiratory failure rabbits were treated with a single intratracheal dose of 100 mg/kg of synthetic surfactant composed of 3% Super Mini-B (S-MB), a SP-B mimic, and/or SP-C33 UCLA, a SP-C mimic, in a lipid mixture (DPPC:POPC:POPG 5:3:2 by weight), the clinical surfactant Infasurf®, a bovine lung lavage extract with SP-B and C, or synthetic lipids alone. End-points consisted of arterial oxygenation, dynamic lung compliance, and protein and lipid content in bronchoalveolar lavage fluid. Potential mechanism of surfactant action for S-MB and SP-C33 UCLA were investigated with captive bubble surfactometry (CBS) assays. Results. All three surfactant peptide/lipid mixtures and Infasurf equally lowered the minimum surface tension on CBS, and also improved oxygenation and lung compliance. In both animal models, the two-peptide synthetic surfactant with S-MB and SP-C33 UCLA led to better arterial oxygenation and lung compliance than single peptide synthetic surfactants and Infasurf. Synthetic surfactants and Infasurf improved lung function further in lavage- than in chemical-induced respiratory failure, with the difference probably due to greater capillary-alveolar protein leakage and surfactant dysfunction after HCl instillation than following lung lavage. At the end of the duration of the experiments, synthetic

surfactants provided more clinical stability in ALI/ARDS than Infasurf, and the protein content of bronchoalveolar lavage fluid was lowest for the two-peptide synthetic surfactant with S-MB and SP-C33 UCLA. Conclusion. Advanced synthetic surfactant with robust SP-B and SP-C mimics is better equipped to tackle surfactant inactivation in chemical ALI than synthetic surfactant with only a single surfactant peptide or animal-derived surfactant.

1 **Synthetic surfactant containing SP-B and SP-C mimics is superior to single-**

2 **peptide formulations in rabbits with chemical acute lung injury**

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22

1   **ABSTRACT**

2   **Background.** Chemical spills are on the rise and inhalation of toxic chemicals may induce

3   chemical acute lung injury (ALI)/acute respiratory distress syndrome (ARDS). Although the

4   pathophysiology of ALI/ARDS is well understood, the absence of specific antidotes besides has

5   limited the effectiveness of therapeutic interventions.

6   **Objectives.** Surfactant inactivation and formation of free radicals are important pathways in

7   (chemical) ALI. We tested the potential of lipid mixtures with advanced surfactant protein B and

8   C (SP-B and C) mimics to improve oxygenation and lung compliance in rabbits with lavage- and

9   chemical-induced ALI/ARDS.

10   **Methods.** Ventilated young adult rabbits underwent repeated saline lung lavages or underwent

11   intratracheal instillation of hydrochloric acid to induce ALI/ARDS. After establishment of

12   respiratory failure rabbits were treated with a single intratracheal dose of 100 mg/kg of synthetic

13   surfactant composed of 3% Super Mini-B (S-MB), a SP-B mimic, and/or SP-C33 UCLA, a SP-C

14   mimic, in a lipid mixture (DPPC:POPC:POPG 5:3:2 by weight), the clinical surfactant

15   Infasurf®, a bovine lung lavage extract with SP-B and C, or synthetic lipids alone. End-points

16   consisted of arterial oxygenation, dynamic lung compliance, and protein and lipid content in

17   bronchoalveolar lavage fluid. Potential mechanism of surfactant action for S-MB and SP-C33

18   UCLA were investigated with captive bubble surfactometry (CBS) assays.

19   **Results.** All three surfactant peptide/lipid mixtures and Infasurf equally lowered the minimum

20   surface tension on CBS, and also improved oxygenation and lung compliance. In both animal

21   models, the two-peptide synthetic surfactant with S-MB and SP-C33 UCLA led to better arterial

22   oxygenation and lung compliance than single peptide synthetic surfactants and Infasurf.

23   Synthetic surfactants and Infasurf improved lung function further in lavage- than in chemical-

1   induced respiratory failure, with the difference probably due to greater capillary-alveolar protein

2   leakage and surfactant dysfunction after HCl instillation than following lung lavage. At the end

3   of the duration of the experiments, synthetic surfactants provided more clinical stability in

4   ALI/ARDS than Infasurf, and the protein content of bronchoalveolar lavage fluid was lowest for

5   the two-peptide synthetic surfactant with S-MB and SP-C33 UCLA.

6   **Conclusion.** Advanced synthetic surfactant with robust SP-B and SP-C mimics is better

7   equipped to tackle surfactant inactivation in chemical ALI than synthetic surfactant with only a

8   single surfactant peptide or animal-derived surfactant.

9

10 **Keywords:** synthetic surfactant, surfactant protein B, surfactant protein C, acute lung injury,

11 lung lavage, hydrochloric acid, ventilated rabbits, oxygenation, lung compliance, captive bubble

12 surfactometry

4

1   **INTRODUCTION**

2   Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are

3   characterized by severe hypoxic respiratory failure and poor lung compliance, mostly caused by

4   lung injury due to pneumonia, aspiration, sepsis and trauma (Bernard et al., 1994; Matthay, Ware

5   & Zimmerman, 2012). Despite advances in respiratory support, morbidity and mortality of

6   ALI/ARDS continue to be elevated due to a lack of efficient therapeutic modalities. Surfactant

7   replacement therapy has long been considered to be a logical pharmacologic approach as the

8   poor lung compliance in ALI/ARDS is associated with surfactant deficiency and inactivation,

9   However, whereas animal-derived surfactant preparations are highly active in preventing and

10   treating respiratory distress syndrome (RDS) in premature infants (Polin & Carlo, 2014), their

11   efficacy in pediatric and adult patients with ALI/ARDS has been utterly disappointing (Willson

12   et al., 2005; Czaja, 2007). After recombinant SP-C based surfactant (Venticute®, Nycomed

13   GmbH, Konstanz, Germany) failed to improve oxygenation in a recent randomized clinical trial

14   because of insufficient surface activity (Spragg et al., 2004 & 2011), doubts have risen about the

15   rationale for exogenous surfactant treatment in ALI/ARDS (Brower & Fessler, 2011;

16   Dushianthan et al., 2012).

17   Chemical spills are frequently in the news and the lung has come under attack from

18   chemical spills and even bioterrorism (Maynard & Tetley, 2004; White & Martin, 2010). The

19   lung's response to inhalation injury of toxic chemicals (chemical ALI) ranges from reactive

20   airways dysfunction syndrome (RADS) to ALI/ARDS with possible fatal outcome (Maynard &

21   Tetley, 2004; Gorguner et al., 2004; White & Martin, 2010). Depending on the toxicant, dose

22   and host factors, cell damage (necrosis or apoptosis) will erode the capillary-alveolar barrier and

23   increase the "leakage" of plasma proteins and water into the interstitium and ultimately into the

1   alveolar spaces. Proteinaceous alveolar edema inactivates lung surfactant and critically affects

2   lung function by reducing lung compliance. Reaction of toxicants with double bonds in

3   unsaturated lipids in cell membranes and lung surface fluids can start a cascade of free radical

4   reactions that further damages cells. Peroxides and carbonyls produced in these reactions may

5   elicit inflammation in the lung via the prostaglandin and leukotriene cascades and magnify and

6   disseminate the toxic injury. The debris of damaged cells and a wide variety of mediators, such

7   as cytokines and chemokines, released by airway epithelium, will attract macrophages and other

8   lung matrix cells and activate various cell surface adhesion molecules, i.e. integrins, on the

9   vascular and respiratory surface. Although the pathophysiology of toxic inhalation injury is well

10   understood (Matthay, Ware & Zimmerman, 2012), specific antidotes to toxic inhalants are not

11   yet available. A potential approach towards chemical ALI, and possibly ALI/ARDS in general,

12   may be offered by the use of a new generation of synthetic surfactant that effectively counteracts

13   surfactant inactivation due to vascular leakage of serum proteins, acute inflammation and

14   oxidative stress (Walther et al., 2000, 2007 & 2010; Curstedt, Calkovska & Johansson, 2013;

15   Johansson et al., 2003).

16   Native **l**ung surfactant is a complex mixture that plays a pivotal role in normal breathing

17   because of its ability to reduce alveolar surface tension to low values and consists of ~80%

18   phospholipids, 10% neutral lipids and 10% proteins (Notter, 2000). Its biophysical activity

19   depends uniquely on the hydrophobic surfactant proteins B (SP-B; 79 residues) and C (SP-C; 35-

20   residues) (Goerke, 1998; Johansson, 1998; Whitsett & Weaver, 2002). Recent work by our

21   group on synthetic SP-B mimics and by the group of Johansson and Curstedt (Karolinska

22   Institute, Stockholm, Sweden) on synthetic SP-C mimics has led to the creation of highly surface

23   active peptide mimics of SP-B and SP-C, i.e. Super Mini-B (S-MB) (Walther et al., 2010) and

1 SP-C33 (Johansson et al., 2003). S-MB is an SP-B mimic that reproduces the topology of the N-

2 and C-terminal domains of SP-B by joining the N-terminal (residues 1-25) and C-terminal

3 (residues 63-78) α-helices with a custom β-turn that replaces SP-B residues 26-62. S-MB has

4 been shown to be highly surface active *in vitro* and *in vivo* (Walther et al., 2010). The function of

5 SP-C is highly dependent on preservation of its transmembrane α-helix in lipids and targeted

6 amino-acid replacements in the α-helix have enabled the development of SP-C33, an SP-C

7 mimic with enhanced α-helicity in lipids and surface activities, resembling those of native SP-C

8 (Johansson et al, 2003; Almlén et al., 2010). Surface activity is not only dependent on the

9 quantity and quality of surfactant peptides in lipid mixtures, but also on the lipid constituents

10 themselves, their interaction with surfactant peptides, and the viscosity of peptide/lipid mixtures

11 (Tanaka et al., 1986; Walther et al., 2005; Seurynck-Servoss et al., 2007). Recent research has

12 led us to formulate advanced SP-B and SP-C peptides in a lipid mixture that mimics the

13 composition of native lung surfactant (Notter, 2000; Walther et al., 2005).

14   Although *in vitro* methods can rapidly provide information about surface activity of

15 experimental surfactant preparations, whole-animal studies are still necessary to test potential

16 treatment modalities in chemical lung injury. Surfactant deficiency induced by repeated saline

17 lung lavages (Ito et al., 1996; Walther et al., 1998) and surfactant dysfunction induced by

18 intratracheal instillation of hydrochloric acid (Chiumello, Pristine & Slutsky, 1999; Brackenbury

19 et al., 2001) in rats and rabbits are established animal models for ALI/ARDS. Both models allow

20 serial measures of arterial blood gases and represent a relatively pure state (over the first 6-9

21 hours or so) of surfactant dysfunction in animals with mature lungs. In this study, we tested the

22 potential of advanced synthetic surfactant preparations to stabilize and improve lung function in

23 adult rabbits with ALI/ARDS induced by lung lavages and chemical exposure.

1   **MATERIALS AND METHODS**

2   *Materials*

3   Peptide synthesis reagents were purchased from Applied Biosystems (Foster City, CA),

4   high performance liquid chromatography (HPLC) solvents from Fisher Chemical Co.

5   (Pittsburgh, PA), and all other chemicals from Sigma Chemical Co. (St. Louis, MO) and Aldrich

6   Chemical Co. (Milwaukee, WI). Dipalmitoylphosphatidylcholine (DPPC), palmitoyloleoyl-

7   phosphatidylcholine (POPC) and palmitoyloleoylphosphatidylglycerol (POPG) were from

8   Avanti Polar Lipids (Alabaster, AL). The clinical surfactant Infasurf® (Calfactant), a bovine

9   lung lavage extract, was a generous gift of Ony Inc (Amherst, NY). Young adult New Zealand

10   White rabbits, weighing 1.0-1.3 kg, were obtained from I.F.P.S. (Norco, CA).

11

12   *Synthesis and Characterization of Surfactant Peptides*

13   S-MB peptide (41 residues, linear sequence NH_2-

14   FPIPLPYCWLCRALIKRIQAMIPKGGRMLPQLVCRLVLRCS-COOH) (Walther et al., 2010)

15   and SP-C33 (NH_2-IPSSPVHLKRLKLLLLLLLLILLLILGALLMGL-COOH)) (Johansson et al.,

16   2003) were synthesized on a Symphony Multiple Peptide Synthesizer (Protein Technologies,

17   Tucson, AZ) with standard FastMoc™ chemistry (Walther et al., 2010). Crude peptides were

18   purified by reverse phase HPLC, molecular weights of the peptides were verified by MALDI-

19   TOF, and α-helicity was determined by FTIR spectroscopy. Disulfide connectivities for S-MB

20   (i.e., Cys-8 to Cys-40 and Cys-11 to Cys-34) were confirmed by mass spectroscopy of enzyme-

21   digested fragments (trypsin and chymotrypsin digestion) (Walther et al., 2010). We renamed the

22   SP-C33 analog produced in our lab as SP-C33 UCLA to distinguish it from SP-C33 made by

23   Chiesi Pharmaceutici SpA (Parma, Italy).

1 *Surfactant Preparations*

2 Synthetic surfactant preparations were formulated by mixing synthetic phospholipids,

3 consisting of 5:3:2 (weight ratio) DPPC:POPC:POPG, with 3% S-MB, 3% SP-C33 UCLA, or

4 1.5% S-MB + 1.5% SP-C33 UCLA. All surfactant preparations were formulated at a

5 concentration of 35 mg phospholipids/ml. The composition of the synthetic phospholipid mixture

6 was based on the lipid composition of native lung surfactant (Notter, 2000; Walther et al, 2005).

7 Infasurf, which contains 35 mg/ml of phospholipids with 1.5% proteins, of which 0.8% is SP-B,

8 was used as positive control and synthetic lipids alone as negative control.

9

10 *Captive Bubble Surfactometry*

11 Adsorption and dynamic surface tension lowering ability of all surfactant preparations

12 were measured with a captive bubble surfactometer at physiological cycling rate, area

13 compression, temperature, and humidity (Walther et al., 2010). We routinely analyze surfactant

14 samples of 1 μl (35 mg phospholipids/ml) in the captive bubble surfactometer and perform all

15 measurements in quadruplicate.

16

17 *Animal studies*

18 The animal studies were reviewed and approved by the Institutional Animal Care and

19 Use Committee of the Los Angeles Biomedical Research Institute at Harbor-UCLA Medical

20 Center (Research Project # 12507). All procedures and anesthesia were in accordance with the

21 American Veterinary Medical Association (AMVA) Guidelines.

22

1   Young adult New Zealand white rabbits (weight 1.0-1.3 kg) received anesthesia with 50

2   mg/kg of ketamine and 5 mg/kg of acepromazine intramuscularly prior to placement of a venous

3   line via a marginal ear vein. After intravenous administration of 1 mg/kg of diazepam and 0.2

4   mg/kg of propofol, a small incision was made in the skin of the anterior neck for placement of an

5   endotracheal tube and a carotid arterial line. After placement of the endotracheal tube, muscle

6   paralysis was induced with intravenous pancuronium (0.1 mg/kg). During the ensuing duration

7   of mechanical ventilation, anesthesia was maintained by continuous intravenous administration

8   of 3 mg/kg/h of propofol and intravenous dosages of 1 mg/kg of diazepam as needed; muscle

9   paralysis was maintained by hourly intravenous administration of 0.1 mg/kg of pancuronium.

10   Heart rate, arterial blood pressures and rectal temperature were monitored continuously

11   (Labchart® Pro, ADInstruments Inc., Colorado Springs, CO, USA). Respiratory function was

12   followed by measurements of arterial pH and blood gases and dynamic lung compliance at 15

13   min intervals. Dynamic lung compliance was calculated by dividing tidal volume/kg body

14   weight by changes in airway pressure (peak inspiratory pressure minus positive end-expiratory

15   pressure) (ml/kg/cm H_2O). Maintenance fluid was provided by a continuous infusion of Lactated

16   Ringer's solution at a rate of 10 ml/kg/h.

17   After stabilization on the ventilator, lung injury was induced by saline lung lavage or

18   intratracheal administration of hydrogen chloride (HCl). Lung lavaging results in loss of active

19   surfactant, whereas HCl instillation leads to epithelial and endothelial damage, lung hemorrhages

20   and copious edema formation resulting in surfactant dysfunction. When the partial pressure of

21   oxygen in arterial blood (PaO$_2$) was >500 torr at a peak inspiratory pressure <15 cm H_2O in

22   100% oxygen (FiO$_2$=1.0), the rabbits underwent repeated saline lung lavages until the PaO$_2$

23   dropped below 100 torr (average 3 lavages of 30 ml of normal saline) or received intratracheal

1   HCl until the PaO$_2$ dropped below 200 torr (average 2 doses of 1.5 ml/kg of 0.1 N HCl, pH 1.0,

2   at 15 min intervals). Edema fluid appearing in the trachea was removed by suctioning. When the

3   PaO$_2$ was stable at <100 torr in lavaged animals (n=39) or the PaO$_2$/FiO$_2$ ratio had reached stable

4   values <200 torr (PaO$_2$/FiO2 <40% of pretreatment values) within 30 min in HCl-exposed

5   animals (n=40), an experimental or positive (Infasurf) or negative (synthetic lipids alone) control

6   surfactant mixture was instilled into the trachea at a dose of 100 mg/kg body weight and a

7   concentration of 35 mg/ml. Group size was 7-9 in the lavaged and 8 in the HCl-exposed rabbits.

8   All rabbits were ventilated using a Harvard volume-controlled animal ventilator (tidal volume

9   7.5 ml/kg, positive end-expiratory pressure of 3 cm H$_2$O, inspiratory/ expiratory ratio of 1:2,

10   100% oxygen, and a respiratory rate to maintain the PaCO$_2$ at ~40 mmHg). Airway flow and

11   pressures and tidal volume were monitored continuously with a pneumotachograph connected to

12   the endotracheal tube and a pneumotach system (Hans Rudolph Inc., Kansas City, MO, USA).

13   Animals were sacrificed 2 h after surfactant administration with an overdose of pentobarbital.

14   End-points were gas exchange (arterial pH, PaCO$_2$ and PaO$_2$), pulmonary mechanics (dynamic

15   lung compliance), and bronchoalveolar lavage fluid proteins and lipids.

16

17   ***Protein and lipid measurements of bronchoalveolar lavage fluid (BALF)***

18   Protein and lipid measurements of bronchoalveolar lavage fluid (BALF) collected during

19   the first lung lavage with 30 ml of normal saline to induce surfactant deficiency (lavaged rabbits

20   only) and the first postmortem lavage (both lavaged and HCl-treated rabbits) are shown in Table

21   1. Protein was measured using the Lowry assay with human albumin as a standard.

22   Phospholipids were measured by extracting BALF samples in chloroform:methanol 2:1 v:v (1 ml

23   of BALF + 4 ml chloroform:methanol), applying the extract to the Fourier transform infrared-

1 attenuated total reflection (FTIR-ATR) plate and drying it before taking a spectrum

2 (Goormaghtigh, Cabiaux & Ruysschaert, 1990).

3

4 *Data analysis*

5   Data are expressed as means ± standard error (SEM). Statistical differences were

6 estimated using t-tests and analyses of variance (ANOVA). Student's t-test was used for

7 comparisons versus control values. Between groups comparisons at various time-points were

8 done by one-way ANOVA and time courses were analyzed with one-way repeated measure

9 ANOVA. A p value <0.05 was considered to indicate a significant difference.

10

11 **RESULTS**

12   S-MB, SP-C33 UCLA and S-MB + SP-C33 UCLA surfactant and Infasurf (positive

13 control) all had very high surface activity in captive bubble experiments and reached minimum

14 surface tension values $\leq$1 mN/m during each of ten consecutive cycles of dynamic cycling (rate

15 of 20 cycles/min, Figure 1). Lipids alone (negative control) reached significantly higher

16 minimum surface tension values of 16 mN/m (p<0.001) than the one- and two peptide/lipid

17 mixtures and Infasurf. Therefore, the relative order for the surfactant activities determined with

18 captive bubble surfactometry for the various preparations were as follows: S-MB surfactant ~

19 SP-C33 UCLA surfactant ~ S-MB + SP-C33 UCLA surfactant ~ Infasurf >> Lipids alone.

20   In the surfactant-deficiency model, induced by repeated lung lavages in young adult

21 rabbits, intratracheal instillation of surfactants with S-MB and/or SP-C33 UCLA and Infasurf

22 quickly improved oxygenation and lung compliance, and continued to be biologically active until

23 the end of the experimental period (Figure 2). S-MB was more active than SP-C33 UCLA, but

1   there was an additive effect of SP-C33 UCLA on S-MB function. The oxygenation and dynamic

2   compliance curves obtained for Infasurf were close to those of S-MB surfactant. Instillation of

3   "Lipids alone" had minimal effects on arterial oxygenation or compliance. The relative order of

4   pulmonary activities for the various surfactant preparations in terms of both oxygenation and

5   compliance was as follows: S-MB + SP-C33 UCLA surfactant > S-MB surfactant ~ Infasurf >

6   SP-C33 UCLA surfactant >> Lipids alone. The differences in oxygenation and compliance

7   between S-MB + SP-C33 UCLA surfactant and S-MB, SP-C33 UCLA and Infasurf were

8   statistically significant starting at 90 min after surfactant instillation, ($p<0.01$) (Figure 2).

9   In the surfactant-dysfunction model, induced by one or more intratracheal instillations of

10   HCl in young adult rabbits, all peptide/lipid mixtures and Infasurf were less effective in

11   improving oxygenation and lung compliance than in the lung lavage model (Figures 2 and 3).

12   Oxygenation and lung compliance continued to deteriorate after intratracheal instillation of

13   synthetic lipids alone. Infasurf outperformed the peptide/lipid mixtures during the first 45 min

14   after intratracheal instillation, but then started to lose activity as shown by deteriorating

15   oxygenation and lung compliance and its surfactant activity was surpassed by the consistent

16   performance of the peptide/lipid mixtures thereafter. The two-peptide surfactant mixture of S-

17   MB and SP-C33 UCLA in DPPC:POPC:POPG lipids finally outperformed the one-peptide

18   mixtures with SMB or SP-C33 UCLA and Infasurf, though they all succeeded in stabilizing and

19   improving lung function after induction of chemical lung injury for the duration of the

20   experiment. Instillation of synthetic lipids alone led to a continuous further deterioration of

21   arterial oxygenation and lung compliance. The relative order of pulmonary activity in terms of

22   both oxygenation and compliance ($\geq$ 90 min) was given as: S-MB + SP-C33 UCLA surfactant >

23   S-MB surfactant ~ SP-C33 UCLA surfactants ~ Infasurf >> Lipids alone. The differences in

1   arterial oxygenation and lung compliance between the S-MB + SP-C33 UCLA surfactant and S-

2   MB surfactant ~ SP-C33 UCLA surfactants ~ Infasurf were statistically significant starting at 90

3   min after surfactant instillation (p<0.01) (Figure 3).

4   Average (±SEM) protein content of BALF (Table 1) in the total group of lavaged animals

5   (n=39) increased almost 10-fold from 286±10 μg/ml in the first 30 ml lavage to induce

6   surfactant-deficiency to 2,776±132 μg/ml in the first postmortem lavage. This increase was least

7   for S-MB+SP-C33 UCLA and S-MB surfactant and highest for synthetic lipids alone. Protein

8   content of BALF in the first postmortem lavage of the 40 HCl-instilled rabbits was higher than in

9   the 39 lavaged rabbits (3,514±143 vs 2,776±137, p<0.001). Rabbits from the HCl group treated

10   with S-MB+SP-C33 UCLA had the lowest and rabbits treated with lipids only had the highest

11   protein values in the postmortem BALF samples among the 5 treatment groups (Table 1).

12   Phospholipid values in BALF of lavaged rabbits also increased almost 10-fold from 10.5±1.2 to

13   98.1±10.6 μg/ml after treatment with each of the 4 surfactant preparations or lipid alone.

14   Phospholipid content of the postmortem lavages of rabbits with HCl-induced ALI was

15   comparable to that of the lavaged animals, although slightly, but statistically significantly, lower

16   in rabbits treated with synthetic peptide surfactants than in Infasurf or lipids alone.

17

18   **DISCUSSION**

19   The success of rescue therapy for chemical-induced ALI using synthetic surfactant with

20   advanced and robust SP-B and SP-C mimics represents a significant breakthrough, as previous

21   experiments have indicated only minimal or no improvement in lung function with animal-

22   derived and first generation synthetic surfactant preparations (Lamm & Albert, 1990;

23   Brackenbury et al., 2001; Zimmermann et al., 2010; Lampland et al., 2013). Lamm & Albert

14

1   (1990) tested Survanta, a modified natural surfactant based on a bovine lung extract, in rabbits

2   lungs after intratracheal injection of HCl, but found no improvement in arterial oxygenation.

3   Brackenbury et al. (2001) treated rabbits pretreated with intratracheal HCl with natural ovine

4   surfactant, bovine lipid extract surfactant and recombinant SP-C surfactant (Venticute) and found

5   none of them effective. Recent studies by Zimmermann et al. (2010) and Lampland et al. (2013)

6   compared intratracheal instillation and aerosol delivery of KL4 surfactant (Surfaxin®, Discovery

7   Laboratories, Warrington, PA) with continuous positive airway pressure (CPAP) only in

8   newborn pigs with acute lung injury after HCl instillation. Both KL4 preparations improved

9   survival, but arterial oxygenation did not increase very much over the surfactant pretreatment

10 values.

11   In a recent collaborative study with Tore Curstedt and Jan Johansson from the

12 Karolinska Institute in Sweden, we examined the *in vivo* activities of Mini-B, i.e. a 'truncated'

13 34-residue predecessor of S-MB without the 7 amino-acid insertion sequence at the N-terminal

14 (Waring et al, 2005), and/or SP-C33 with synthetic lipids in preterm newborn rabbits (Almlén et

15 al., 2010). Treatment with either Mini-B or SP-C33 led to increased tidal and lung gas volumes,

16 and combination treatment with these surfactant protein mimics demonstrated an additive effect

17 in this validated animal model for neonatal respiratory distress. These results and the current data

18 in lavaged and HCl-treated young adult rabbits indicate that synthetic surfactants containing

19 analogs of both SP-B and SP-C may be superior to single-peptide surfactants in the treatment of

20 RDS and ALI/ARDS.

21   The BALF findings indicate that treatment with an advanced two-peptide synthetic

22 surfactant led to reduced protein values in the lavage fluid than those for single peptide synthetic

23 surfactant or animal-derived surfactant with both SP-B and SP-C. This finding suggests that a

1 more advanced and robust synthetic surfactant has the potential to diminish capillary-alveolar

2 protein leakage and thereby reduce surfactant inhibition.

3   Animal models have their advantages and limitations. Lung lavage and HCl instillation

4 each result in significant hypoxemia without hemodynamic effects that are fairly stable over at

5 least the first 6-9 hours (Rosenthal et al., 1998). In our hands mortality in the lung lavage model

6 is less than the HCl-model, even though our primary goal of repeated lavages was to decrease

7 arterial PO_2 to values <100 torr and we accepted a higher limit (PaO_2 <200 torr) after HCl

8 instillation because values <80 torr were associated with a quick demise. In fact, the average

9 PaO_2 values after lung lavage were 53 torr (10% of the original values) and 117 torr (22% of

10 original values) after HCl treatment. Lung compliance corresponded with oxygenation, lung

11 lavage decreased lung compliance by 51% versus 39% after HCl treatment. These differences

12 can be explained by a lesser degree of capillary-alveolar protein leakage after lung lavage than

13 acid treatment that not only results in loss of active surfactant, but also leads to epithelial and

14 endothelial damage, lung hemorrhages and copious edema formation resulting in severe

15 surfactant dysfunction.

16

17 **CONCLUSIONS**

18 As opposed to animal-derived (Infasurf) or first generation synthetic (Surfaxin or Venticute)

19 surfactant preparations, rescue therapy with a second generation synthetic (S-MB and SP-C33

20 UCLA in a synthetic three-lipid mixture) was highly effective in stabilizing and improving

21 oxygenation and lung compliance in rabbits with chemical-induced lung injury.

22

23

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1   **Table 1:** Protein and phospholipid values (µg/ml) in BALF obtained during the first lavage

2   to induce surfactant deficiency and the first postmortem lung lavage in lavaged and HCl-

3   treated (postmortem BALF only) rabbits. Data are shown as mean ± SEM.

4

Surfactant	Protein (ug/ml) ± SEM		Phospholipids (ug/ml) ± SEM	
	1st BALF	Postmortem BALF	1st BALF	Postmortem BALF
Lung lavaged rabbits (n=7-8 per group)				
S-MB+SP-C33 UCLA	268±12	2,124±130	10.1±2.7	100.5±17.8
S-MB	302±21	2,358±65	10.8±2.8	96.3±33.0
SP-C33 UCLA	297±27	2,503±146**	9.4±1.9	101.2±15.4
Infasurf	278±22	2,648±137**	11.2±2.8	94.9±12.1
Lipids alone	285±26	4,062±230*	11.4±2.4	105.9±5.5
HCl-instilled rabbits (n=8 per group)				
S-MB+SP-C33 UCLA		2,531±176		83.9±6.5
S-MB		3,337±228**		87.2±5.4
SP-C33 UCLA		3,203±235**		98.3±2.0
Infasurf		3,874±172**		103.9±3.5***
Lipids alone		4,623±224*		105.9±2.7***

5   *: $p<0.01$ vs all other surfactant preparations

6   **: $p<0.05$ vs S-MB+SP-C33 UCLA surfactant

7   ***: $p<0.05$ vs S-MB+SP-C33 UCLA and S-MB surfactant

1 **LEGENDS**

2 **Figure 1:** Surface activity of synthetic lung surfactants, clinical surfactant, and synthetic lipids

3 only on the captive bubble surfactometer. Minimum and maximum surface tension values are

4 plotted for synthetic lipids with 3% (weight ratio) Super Mini-B (S-MB), 3% SP-C33 UCLA or

5 1.5% S-MB + 1.5% SP-C33 UCLA, clinical surfactant (Infasurf), and synthetic lipids alone.

6 Synthetic lipids are 5:3:2 (weight ratio) DPPC:POPC:POPG. Data are shown as mean ± SEM of

7 n=4.

8

9 **Figure 2:** Arterial oxygenation and dynamic compliance in surfactant-treated, ventilated rabbits

10 with ARDS induced by repeated *in vivo* lavage. Arterial partial pressure of oxygen (PaO_2 in torr)

11 and dynamic compliance (ml/kg/cm H_2O) are shown as a function of time for the 5 groups of 7-8

12 ventilated rabbits treated with experimental surfactant at time 0, when PaO_2 had dropped from

13 >500 torr to <100 torr after standardized lung lavages. Rabbits treated with synthetic lung

14 surfactants (synthetic lipids + 3% Super Mini-B [S-MB], 3% SP-C33 UCLA or 1.5% S-MB +

15 1.5% SP-C33 UCLA) and clinical surfactant (Infasurf) as positive and synthetic lipids alone as

16 negative control. Synthetic lipids are 5:3:2 (weight ratio) DPPC:POPC:POPG. Data are shown as

17 mean ± SEM of groups of 7-8 rabbits.

18

19 **Figure 3:** Arterial oxygenation and dynamic compliance in surfactant-treated, ventilated rabbits

20 with ALI induced by intratracheal instillation of 0.1 N hydrogen chloride (HCl). Arterial partial

21 pressure of oxygen (PaO_2 in torr) and dynamic compliance (mL/kg/cm H_2O) are shown as a

22 function of time for the 5 groups of 8 ventilated rabbits treated with experimental surfactant at

23 time 0, when PaO_2 had dropped below 40% of the starting value after HCl instillation. Rabbits

23

1   were treated with synthetic lung surfactants (synthetic lipids + 3% Super Mini-B [S-MB], 3%

2   SP-C33 UCLA, or 1.5% S-MB + 1.5% SP-C33 UCLA) and clinical surfactant (Infasurf) as

3   positive and synthetic lipids alone as negative control. Synthetic lipids are 5:3:2 (weight ratio)

4   DPPC:POPC:POPG. Data are shown as mean ± SEM of groups of 8 rabbits.
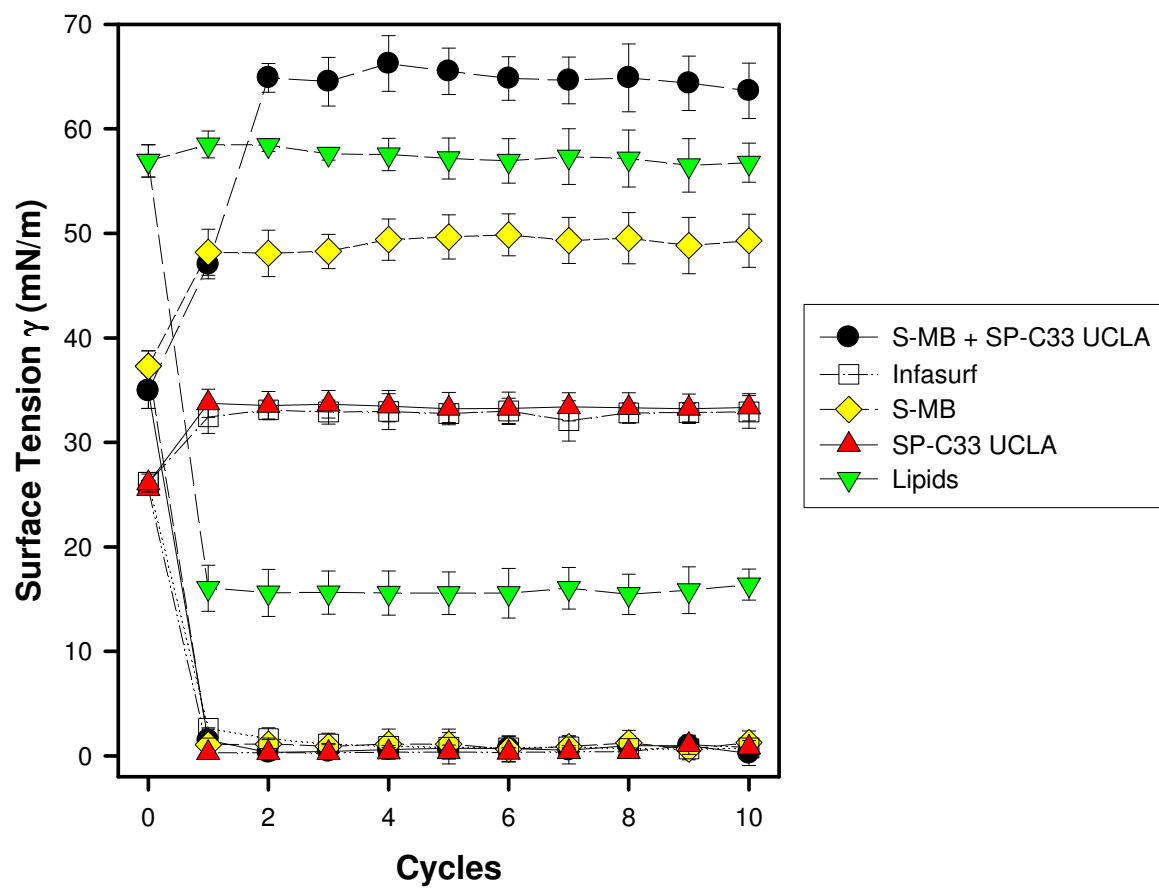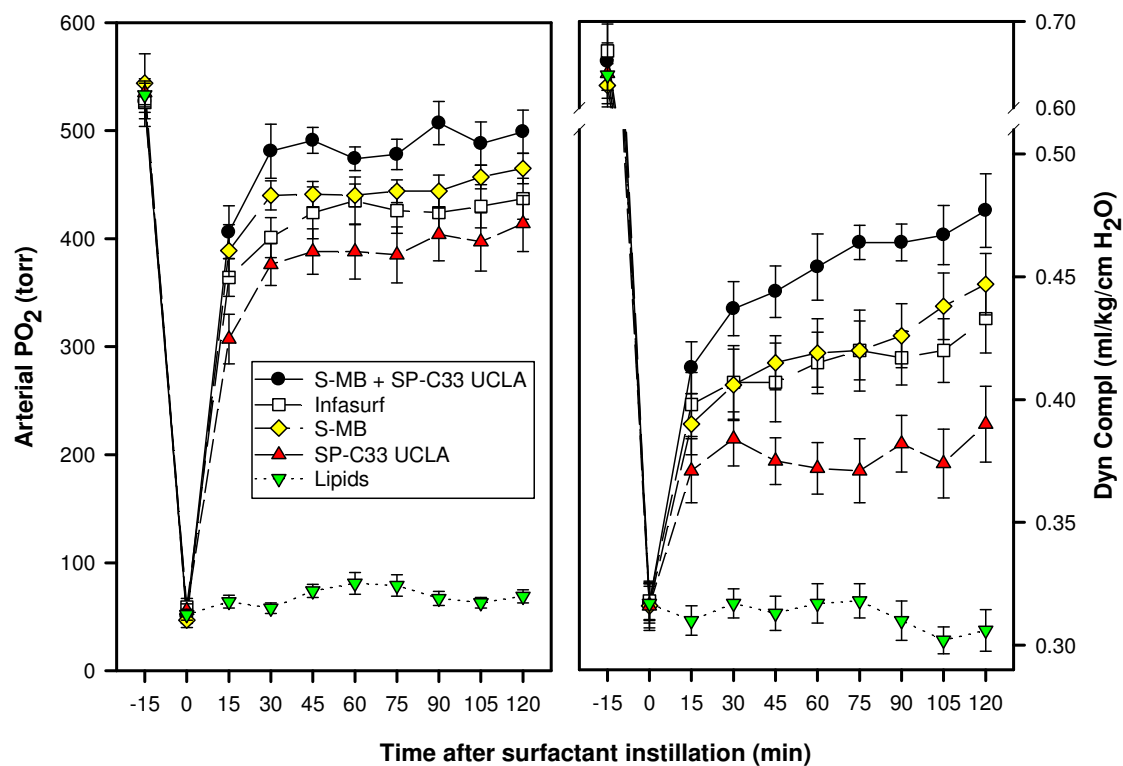
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Figure 1:

Figure 2:

Figure 3: