Surfactant Inhibition in Acute Respiratory Failure

Consequences for Exogenous Surfactant Therapy

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Eijking, Eric P.

Surfactant inhibition in acute respiratory failure: consequences for exogenous surfactant therapy / Eric P. Eijking. - [S.1. : s.n.]. - I11.
Thesis Rotterdam. - With ref. - With summary in Dutch.
ISBN 90-9005873-7
NUGI 743
Subject headings: surfactant inhibition.

SURFACTANT INHIBITION IN ACUTE RESPIRATORY FAILURE

Consequences for Exogenous Surfactant Therapy

SURFACTANT INHIBITIE IN ACUUT RESPIRATOIR FALEN

Consequenties voor Exogeen Surfactant Therapie

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof. Dr. C.J. Rijnvos
en volgens besluit van het college van dekanen.

De openbare verdediging zal plaatsvinden op
woensdag 28 april 1993 om 13.45 uur

door

Eric Paul Eijking
geboren te Hilversum

PROMOTIECOMMISSIE:

Promotor:	Prof. Dr. B. Lachmann
Overige leden:	Prof. Dr. H.A. Bruining
	Prof. Dr. W. Erdmann
	Prof. Dr. C. Hilvering

Dit proefschrift werd bewerkt binnen de afdeling Anaesthesiologie van de Erasmus Universiteit Rotterdam.

The studies presented in this thesis were financially supported by the Dutch Foundation for Medical Research (SFMO).
Additional financial support for printing was received from Boehringer Ingelheim Zentrale GmbH, Germany and Dr. Karl Thomae GmbH, Germany.


Dit proefschrift werd gedrukt door Haveka B.V. te Alblasserdam.

Aan Karin

Aan mijn ouders

CONTENTS

PREFACE

The neonatal respiratory distress syndrome (RDS) is characterized by immaturity of the lung, resulting in relative or absolute absence of pulmonary surfactant. Worldwide, neonates suffering from RDS have been treated successfully with exogenous surfactant preparations. Currently, exogenous surfactant administration has been accepted as a valuable treatment for this syndrome. Nevertheless, many questions on exogenous surfactant treatment remain unanswered.

It has been observed that some infants did not respond to, or had only transient improvement after, a single dose of exogenous surfactant. In other studies it was observed that better clinical outcome was seen in infants treated with either a higher dose of surfactant, or after repeated surfactant substitution. It was argued that exogenous surfactant, after intratracheal substitution, was gradually inhibited by surfactant inhibitors present in the terminal lung units. By giving more exogenous surfactant this inhibition was abolished, resulting in improved lung function.

The adult respiratory distress syndrome (ARDS) is characterized by acute respiratory failure (ARF), and high-permeability edema fluid accumulating in the alveolar spaces. From experimental and clinical studies it is known that the pulmonary surfactant system is also disturbed in this syndrome. Recently, a few patients suffering from ARDS have been treated with exogenous surfactant. Although these case reports are poorly documented, the best results were observed in patients treated with high doses of exogenous surfactant.

The work presented in this thesis focuses on the mechanisms of inhibition of surfactant function in different animal models of acute respiratory failure. To overcome this inhibition, different treatment modalities with an exogenous surfactant preparation will be presented.

OVERVIEW OF THE STUDY

Chapter 1 addresses the pulmonary surfactant system. The composition of pulmonary surfactant and its functions are explained. Special attention is given to exogenous surfactant therapy in neonates suffering from respiratory distress syndrome (RDS); clinical studies on exogenous surfactant therapy in neonatal RDS are summarized (see Appendix A).

In *Chapter 2* involvement of the pulmonary surfactant system in acute respiratory failure (ARF) in the adult respiratory distress syndrome (ARDS) is described. Animal models on ARF are presented, with special reference to models used in our laboratory and the effect of exogenous surfactant therapy in these models. Also, the first case reports on exogenous surfactant therapy in patients suffering from ARDS are discussed (see Appendix B).

It has been proposed that in patients suffering from ARF/ARDS, the surfactant function is inhibited by plasma-derived proteins present in the intra-alveolar space. It has also been suggested that in patients not responding to a single dose of exogenous surfactant (so-called "non-responders"), this surfactant was inhibited by these proteins. *Chapter 3* presents a study in which the inhibitory capacity of both high-permeability edema fluid and homologous plasma is investigated both in vivo and in vitro.

In *Chapter 4* we investigate the amount of exogenous surfactant needed to overcome the inhibitory capacity of heterologous (human) plasma in rats suffering from ARF after whole lung lavage.

It is known that aspiration of gastric contents leads to protein-rich pulmonary edema fluid, containing inhibitors of surfactant function. In clinical practice it is known that ARF caused by massive aspiration of gastric contents is difficult to treat. In *Chapter 5*, in a model for massive aspiration of hydrochloric acid (HCl), the influence of exogenous surfactant instillation at different time intervals post-HCl aspiration on lung function is investigated. It is demonstrated that respiratory failure can be prevented when exogenous surfactant is given before deterioration of lung function (i.e. within 10 min after HCl aspiration), whereas after development of ARF exogenous surfactant cannot restore gas exchange. For this reason, the study in *Chapter 6* is designed to investigate the optimal way to treat rats suffering for a longer period from ARF due to HCl

aspiration with exogenous surfactant. Exogenous surfactant is given 1) as a bolus, 2) after removal of pulmonary edema fluid with bronchoalveolar lavage (BAL), or 3) BAL with a diluted surfactant suspension. It is demonstrated that lung function can be restored after edema fluid is removed prior to exogenous surfactant instillation. Also, gas exchange can be restored after BAL with the diluted surfactant suspension, probably due to simultaneous removal of surfactant inhibitors and surfactant treatment.

In the acute phase of pneumonia, protein-rich edema fluid accumulates in the alveolar space. Recently we have demonstrated that exogenous surfactant could restore lung function in rats and mice suffering from pneumonia due to Sendai virus and influenza A virus infection, respectively. In pneumocystis carinii pneumonia (PCP) the pulmonary surfactant system is also involved. In the study discussed in *Chapter 7*, rats suffering from ARF due to PCP are successfully treated with a bolus of exogenous surfactant.

As natural exogenous surfactants contain proteins foreign to the patient, it seems likely that formation of antibodies to these proteins can be observed. In the study presented in *Chapter 8* a monoclonal antibody is raised against a hydrophobic surfactant-associated protein (11 kDa). It is demonstrated that a mixture of this monoclonal antibody and exogenous surfactant is not able to restore lung function in rats suffering from ARF after whole lung lavage. Although surfactant-antisurfactant immune complexes have been detected in neonates with RDS, no effect on exogenous surfactant therapy has been reported. These results also indicate the importance of the 11 kDa surfactant-associated protein for exogenous surfactant function.

CHAPTER 1

PULMONARY SURFACTANT: BASIC PHYSIOLOGY AND ITS USE FOR REPLACEMENT THERAPY

B. Lachmann and E.P. Eijking

In: Drug targeting and delivery. Concepts in dosage form design. H.E. Junginger (ed.). Ellis Horwood Limited. New York, 1992, pp 129-154

HISTORY

In 1929, von Neergaard wrote *"It may be possible that the surface tension of the alveoli is diminished by concentration of surface-active substances against other physiologic solutions"* (77). He was referring to the existence of a surface film in the alveoli and his assumption was based on the following observations. Neergaard measured pressure-volume diagrams from human and animal lungs, first filling them with air and then with liquid. The surprising result was that the pressure necessary for filling the lung with liquid was only half the pressure necessary for filling the lung with air. His explanation of this remarkable difference was based on the assumption that in each alveolus there must be a barrier between air and fluid (such as the wall of a soap-bubble) with a tendency to diminish its size according to the law of Laplace ($P=2\gamma/r$, in which P = pressure to stabilize a bubble/alveolus; γ = surface tension at air-liquid interface; and r = radius of the bubble/alveolus). The amount of retraction pressure for the lung is larger than the retraction force of the elastic fibres. By filling the alveoli with liquid, the air-liquid barrier is replaced by a liquid-to-liquid barrier without any surface tension. The retraction pressure, measurable in the fluid-filled lung is, therefore, equal to the retraction force of the elastic fibres. The same procedure for determining the influence of surface tension on the overall retraction of the lung was later reported by other scientists, and is now a well-established method.

In 1955 Pattle (60) showed that bubbles resulting from lung edema, as well as bubbles squeezed from lung cut, are very stable. Pattle assumed that the walls of these bubbles consist of surface-active materials and that their stability depends on the quantity and quality of surfactant phospholipids. In 1957 Clements (6) was the first to investigate lung extracts in a Wilhelmy-balance and demonstrated that these extracts had, in contrast to detergents or plasma, genuine hysteresis properties.

Avery and Mead (1) were the first, in 1959, to draw attention to the possibility that lungs of infants with respiratory distress syndrome (RDS) may have abnormal surface tension properties, due to a deficiency in lung surfactant.

BIOCHEMICAL COMPOSITION

Biochemical characterization has shown that surface-active phospholipids, proteins and mucopolysaccharides are the main constituents of the surfactant system of the lung. Generally, between 80% and 90% of surfactant is phospholipid. The major representative (70% to 80%) of surfactant phospholipids is phosphatidylcholine (PC) and about 60% of the PC molecules contain two saturated acyl chains. This disaturated PC (DSPC) is largely dipalmitoylphosphatidylcholine (DPPC). In most adult mammalian species, phosphatidylglycerol (PG) is the second most abundant phospholipid class, accounting for up to 10% of total surfactant lipid. Surfactant contains, in addition, small proportions of phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE) and sphingomyelin (for review see [76]).

Phospholipids are synthetized in the type II alveolar cells. They are stored in these cells as so-called lamellar bodies, before being released to the surface of the alveoli. The surfactant lipids spread as a monolayer at the air-liquid boundary. They reduce the net contractile force of the alveolar surface, thus preventing the airspaces from collapsing at low lung volumes.

Besides phospholipids, surfactant also contains specific surfactant-associated proteins. At least four surfactant-associated proteins (SP-A, B, C and D) are produced by type II cells. These proteins have, apart from functions such as the regulation of phospholipid metabolism, an important function in the formation of the surfactant monolayer (76). It has been demonstrated that for optimal function of the lipids the presence of a few small proteins is a prerequisite for optimal in vivo function of artificial surfactant and surfactant extracted from mammalian lungs (76).

FUNCTION OF THE SURFACTANT SYSTEM

Mechanical stabilization of the lung

The integrity of the surfactant system of the lung is a prerequisite for normal breathing with the least possible effort. The surfactant system acts by decreasing surface tension of the interface between alveoli and air. This provides an explanation as to why we have to generate a "negative" pressure of only 5-10 cmH_2O during each inspiration; in the

absence of surfactant the surface tension at the air-liquid interface would be that of plasma and the pressure needed to maintain lung aeration would be 25-30 cmH_2O (depending on the radius of the alveoli). This is a well-known problem in patients with respiratory failure.

In alveoli with different radii an equal lowering of surface tension would not, however, produce stabilization of the alveolar system. It would instead, according to the law of Laplace, lead to the collapse of the smaller bubbles or alveoli, and to their emptying into the larger bubbles/alveoli. Since alveoli in vivo do not exhibit such behavior, one can conclude that the second quality of the alveolar lining layer is that it can change the surface tension in a manner related to the size of the alveoli.

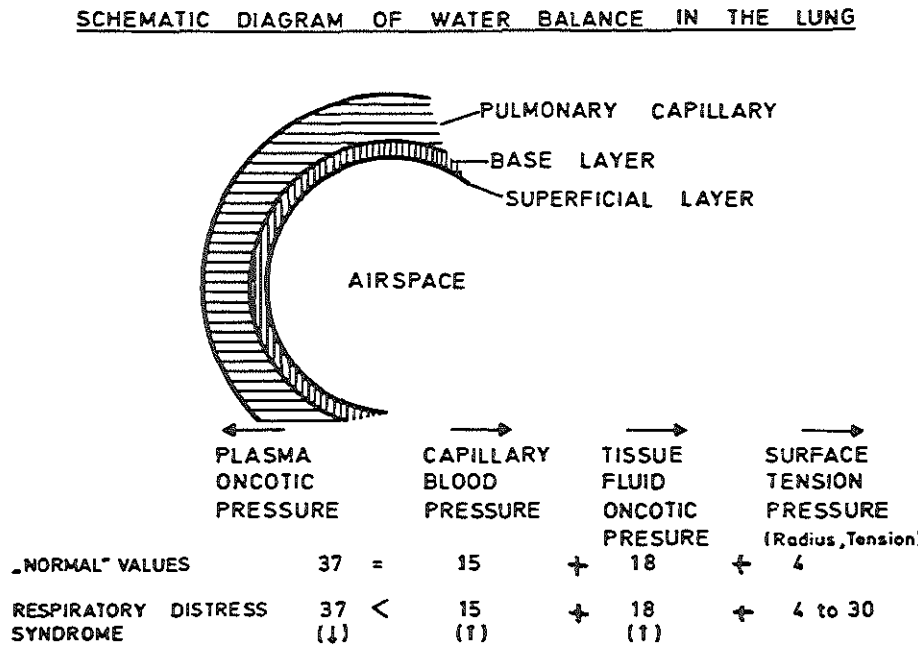


Figure 1. Simplified schematic diagram representing the factors influencing the fluid balance in the lung.

Surfactant as anti-edema factor

Another function of the pulmonary surfactant system is stabilization of the fluid balance in the lung, and protection against edema. The normal plasma oncotic pressure of 37 cmH_2O is opposed by the oncotic pressure of interstitial proteins of 18 cmH_2O, the capillary hydrostatic pressure of 15 cmH_2O, and by the surface tension conditioned suction of 4 cmH_2O (Fig. 1).

In general, alveolar flooding will not occur as long as the suction force in the pulmonary interstitium exceeds the pressure gradient generated by the surface tension in the alveolar air-liquid interface. Since this pressure gradient is inversely related to the radius of the alveolar curvature there is, for each combination of interstitial resorptive force and average surface tension, a critical value for surface tension and for alveolar radius, below which alveolar flooding occurs (20).

Surfactant and local defence mechanisms

It has been suggested that the surfactant system is involved in the local defence mechanisms of the lung. In the past it has been demonstrated that alveolar macrophages ingest bacteria (and destroy them intracellularly) only in the presence of sufficient surface-active material (29,30). In this context surfactant seems to reduce the surface forces of bacterial membranes and it is also an energy-rich substrate which supports the macrophages' high rate of metabolism.

Recently we have demonstrated that the pulmonary surfactant system may also be involved in protecting the lung against its own mediators (e.g. angiotensin II) and in protecting the cardiocirculatory system against mediators produced by the lung.

Surfactant and airways stabilization

As early as 1970, Macklem et al (46) called attention to the significance of bronchial surfactant for the stabilization of the peripheral airways and hinted that lack of stabilization may cause airway obstruction or collapse of the small bronchi with air trapping. This has been proved in our laboratory in an animal model where the bronchial surfactant was selectively destroyed (39). It was demonstrated that the pressure to open up the collapsed bronchi is about 20 cmH_2O.

Besides its role in mechanical stabilization, bronchial surfactant also has a transporting function for mucus and inhaled particles. This has been proven, in vitro, in a study showing that particles on a surface film move in one direction only if the surface film is compressed and dilated, comparable to the compression and expansion during expiration and inspiration (39). Furthermore, bronchial surfactant also acts as an antiglue factor preventing the development of large adhesive forces between mucus particles, as well as between mucus and the bronchial wall (Fig. 2) (63).

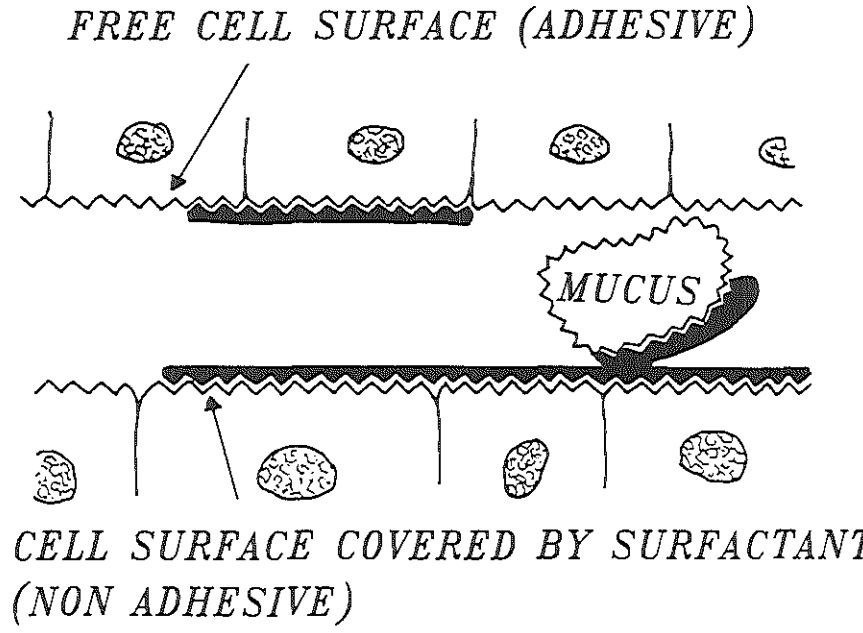


Figure 2. Schematic diagram demonstrating the interaction between mucus and airway. Note that there will be non-adhesive forces only if the mucus particle and the airways are covered with surfactant (thick solid line).

A further possible function of bronchial surfactant, which to date has scarcely been discussed, is its masking of receptors on smooth muscle with respect to substances which

induce contraction and could lead to airway obstruction. We have recently demonstrated that lining the airway with surfactant in ovalbumin-sensitized guinea pigs prevented significant bronchial obstruction during antigen challenge in these animals. This means that the bronchial surfactant could also be involved in asthma (40). This is further supported by the fact that drugs used in the treatment of asthma (corticosteroids and betamimetics) lead to a release of surfactant.

FUNCTIONAL CHANGES DUE TO A "DISTURBED" SURFACTANT SYSTEM

When considering the main physiologic functions of the alveo-bronchial surfactant system it can easily be understood that alteration in its functional integrity will lead to:

- decreased lung distensibility (decreased lung compliance) and thus increased work of breathing and increased oxygen demand by the respiratory muscles
- atelectasis and decreased functional residual capacity (FRC)
- transudation of plasma into the interstitium and into the alveoli with decreased diffusion for oxygen and CO_2
- inactivation of the surfactant system by plasma proteins and specific surfactant inhibitors
- hypoxemia and respiratory acidosis
- metabolic acidosis secondary to increased production of organic acids under anaerobic conditions
- enlargement of functional right-to-left shunt due to perfusion of non-ventilated alveoli
- decreased production of surfactant as a result of hypoxemia, acidosis and hypoperfusion.

This will finally lead to a vicious circle and the lung will fail as a gas exchange organ (Fig. 3) (44).

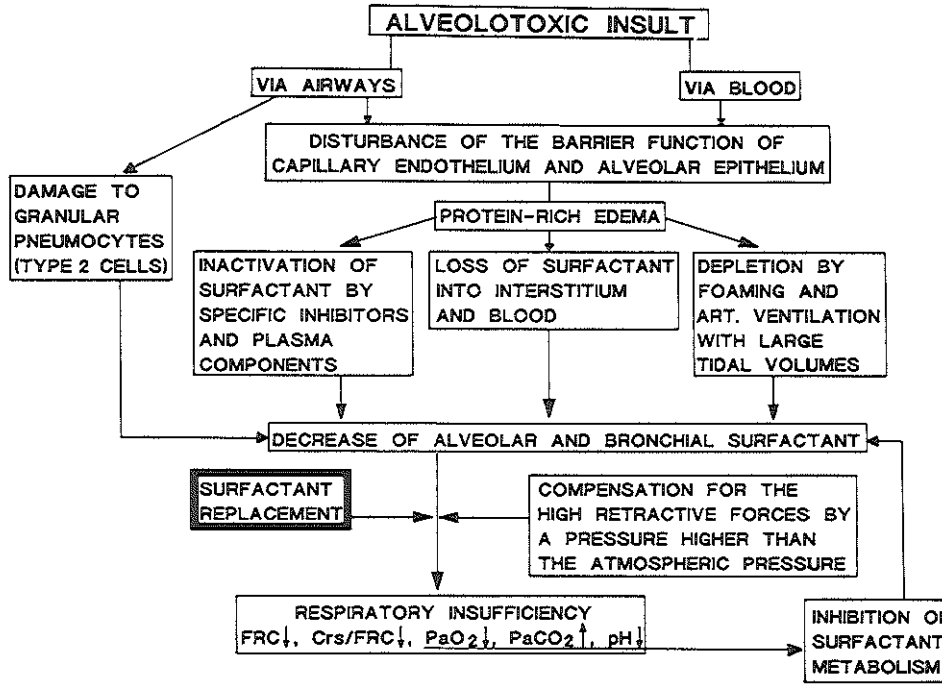


Figure 3. Pathogenesis of ARDS with special reference to the surfactant system, including suggestions to compensate for a damaged surfactant system.

SURFACTANT REPLACEMENT THERAPY

Three decades of active research have resulted in the probability that surfactant treatment of surfactant deficiency states will soon be generally possible.

Exogenous surfactant therapy offers great promise for reducing the severity, morbidity and early mortality attributable to RDS. The RDS remains a major cause of neonatal mortality; it is responsible for approximately one neonatal death per 1000 live births and it contributes to the number of infants with chronic pulmonary sequelae and neurodevelopmental handicaps, emphasizing the need to make efficacious therapies

available.

Soon after the identification of DPPC as the principal surface-active component of surfactant and the recognition in 1959 that saline extracts from lungs of infants who died of RDS had abnormal surface properties (1), aerosols of DPPC were used to treat RDS, but with little beneficial effect (5,66) (Tables 1 and 2: see Appendix A). However, during the 1970s, Enhorning and Robertson (10) developed a reliable experimental basis for the concept that RDS could be treated with exogenously administered surfactant. The idea was first successfully tested in infants by Fujiwara and coworkers in 1980 (13) and, subsequently, the number of clinical studies have increased exponentially. Clinical trials have been designed either as rescue or as prophylactic studies (Tables 1 and 2: see Appendix A).

The terminology used to describe various surfactants in the experimental and clinical literature is inconsistent. There are four general categories of surfactants that are being evaluated for clinical use:

Natural surfactant. The surfactant that can be removed from fresh alveolar washes or from amniotic fluid by simple centrifugation and/or filtration procedures designed to recover the large surface-active aggregates of surfactant (23,31). Such natural surfactant contains more protein (and all categories of surfactant-associated proteins) than the more highly purified surfactant, but it can be recovered in the large amounts necessary for treatment protocols. Surfactant prepared after lipid extraction procedures are specifically excluded from this category since the native surfactant aggregates are disrupted.

Modified natural surfactants are made from species homologous or heterologous lungs, or alveolar lavage. Such surfactants are prepared by extraction of lipids followed by selective addition and/or removal of compounds, sterilization and suspension procedures designed to restore the desired surface properties (8,15,75). The final surfactant may contain as much as 1% hydrophobic surfactant-associated proteins (SP-B and SP-C) (78). Modified natural surfactants are designed to reproduce or improve upon the characteristics of natural surfactant, to decrease protein contamination and to assure sterility.

Artificial surfactants are those surfactans made from a mixture of synthetic compounds that may or may not be normal components of natural surfactant. The most studied artificial surfactant is a mixture of 70% DPPC and 30% PG (54). While both phospholipids are present in natural surfactant, the proportions differ and the acyl groups of PG are different from those found in the PG of natural surfactant. Another artificial surfactant contains compounds foreign to natural surfactant, such as hexadecanol and tyloxapol. There are no surfactant-associated proteins present in these artificial surfactants.

Synthetic natural surfactants are just beginning to be a possibility. With the recent isolation and characterization of the surfactant-associated proteins, synthetic natural surfactants are being tested. In the near future, such surfactants will be reconstructed in vitro from surfactant-associated proteins synthesized using molecular biology techniques, and from mixtures of lipids and phospholipids.

CONCLUSIONS

Initial reports suggest that surfactant treatments will simplify care sufficiently that hospital stay and thus cost of care may be decreased (11). Many practical aspects of the treatment of infants with surfactant have not been evaluated. The dose, the most effective method of administration and the benefits from repetitive doses are not well defined. While a number of surfactants have been evaluated clinically, some were "home-made" and not well standardized. Different surfactants have not been compared directly in clinical trials. The surfactant with the best clinical response may not be a surfactant that can be easily standardized and prepared in bulk as a pharmaceutical. Practical considerations of formulation and licensure will, in part, dictate which surfactant will ultimately be available for clinical use.

Surfactant therapy should not only be beneficial to those infants in need of supplemental surfactant, but also indirectly to infants who suffer secondary to surfactant deficiency. Other uses of surfactant are now being considered. Experimental models of lung injury in adult animals such as saline lavage (2,33), oxygen injury (47), viral and bacterial pneumonia, oxygen free radicals, anti-lung serum and pulmonary edema

(38,41,42,43) (see Chapter 2), together with first clinical trials (44) suggest that there is a role for surfactant in the treatment of the adult respiratory distress syndrome (ARDS). Surfactant treatments will become a major advance in the care of preterm infants with severe RDS, and may ultimately prove useful in other lung diseases.

REFERENCES

1. Avery ME, Mead J. Surface properties in relation to atelectasis and hyaline membrane disease. Am J Dis Child 1959; 97: 517-523
2. Berggren P, Lachmann B, Curstedt T, Grossmann G, Robertson B. Gas exchange and lung morphology after surfactant replacement in experimental adult respiratory distress syndrome induced by repeated lung lavage. Acta Anaesthesiol Scand 1986; 30: 321-328
3. Bloom BT. Human surfactant and calf lung surfactant extract: moderation of respiratory distress in preterm infants by a single prophylactic dose in a randomized and controlled clinical trial. In: Lachmann B (ed) Surfactant Replacement Therapy in Neonatal and Adult Respiratory Distress Syndrome. Berlin, Heidelberg, Springer-Verlag 1988: 150-157
4. Charon A, Taeusch T, Fitzgibbon C, Smith GB, Treves ST, Phelps DS. Factors associated with surfactant treatment in infants with severe respiratory distress syndrome. Pediatrics 1989; 83: 348-354
5. Chu J, Clements JA, Cotton EK, Klaus MH, Sweet AY, Tooley WH. Neonatal pulmonary ischemia. Pediatrics 1967; 40: 709-782
6. Clements JA. Surface tension of lung extracts. Proc Soc Exp Biol Med 1957; 95: 170-172
7. Davis JM, Veness-Meehan K, Notter RH, Bhutani VK, Kendig JW, Shapiro LD. Changes in pulmonary mechanics after the administration of surfactant to infants with respiratory distress syndrome. N Engl J Med 1988; 319: 476-479
8. Egan EA, Notter RH, Kwong MS, Shapiro DL. Natural and artificial lung surfactant replacement therapy in premature lambs. J Appl Physiol 1983; 55: 875-883
9. Egan EA, Kwong MS. Clinical results of a multicenter open trial of Infasurf. In: Ross Laboratories special conference: Hot topics '88 in Neonatology 1988: 86-96
10. Enhorning G, Robertson B. Lung expansion in the premature rabbit fetus after tracheal deposition of surfactant. Pediatrics 1972; 50: 58-66
11. Enhorning G, Shennan A, Possmayer F, Dunn M, Chen CP, Milligan J. Prevention of neonatal respiratory distress syndrome by tracheal instillation of surfactant: a randomized clinical trial. Pediatrics 1985; 76: 145-153
12. Friedman ZVI, Doody M. Artificial surfactant: a therapeutic trial in infants with hyaline membrane disease. Pediatr Res 1982; 16: 287A
13. Fujiwara T, Maeta H, Chida S, Morita T, Watabe Y, Abe T. Artificial surfactant therapy in hyaline-

membrane disease. Lancet 1980; 1: 55-59

14. Fujiwara T. Tracheal instillation of artificial surfactant for the treatment of hyaline membrane disease. In: Newer management of hyaline membrane disease. Ann Nestlé 1981: 48: 24-29

15. Fujiwara T. Surfactant replacement in neonatal RDS. In: Robertson B, Van Golde LMG, Batenburg JJ (eds) Pulmonary Surfactant. Amsterdam, Elsevier 1984: 479-503

16. Fujiwara T, Onishi M, Nanby H. Surfactant supplementation treatment of neonatal respiratory distress syndrome. Shonika Rinsho 1987; 40: 549-568

17. Fujiwara T, Konishi M, Chida S, Maeta H. Factors affecting the response to a postnatal single dose of a reconstituted bovine surfactant (Surfactant TA). In: Lachmann B (ed) Surfactant Replacement Therapy in Neonatal and Adult Respiratory Distress Syndrome. Berlin, Heidelberg, Springer-Verlag 1988: 91-107

18. Gitlin JD, Soll RF, Parad RB, Horbar JD, Feldman HA, Lucey JF, Taeusch HW. Randomized controlled trial of exogenous surfactant for the treatment of hyaline membrane disease. Pediatrics 1987; 79: 31-37

19. Gortner L, Pohlandt F, Bartmann P, Disse B, Weller E. Surfactant replacement with SF-RI 1 in premature infants with respiratory distress syndrome: a clinical pilot study. In: Lachmann B (ed) Surfactant Replacement Therapy in Neonatal and Adult Respiratory Distress Syndrome. Berlin, Heidelberg, Springer-Verlag 1988: 133

20. Guyton AC, Moffatt DS, Adair TA. Role of alveolar surface tension in transepithelial movement of fluids. In: Pulmonary Surfactant, Robertson B, van Golde LMG, Batenburg JJ (eds) Pulmonary Surfactant. Elsevier, Amsterdam, 1984: 171-185

21. Halliday HL, McClure G, Reid MMc, Lappin TRJ, Meban C, Thomas PS. Controlled trial of artificial surfactant to prevent respiratory distress syndrome. Lancet 1984; 1: 476-478

22. Hallman M, Schneider H, Merritt TA, Gluck L. Human surfactant substitution. Pediatr Res 1982; 16: 691A

23. Hallman M, Merritt TA, Schneider H, Epstein BL, Mannino F, Edwards DK, Gluck L. Isolation of human surfactant from amniotic fluid and a pilot study of its efficacy in respiratory distress syndrome. Pediatrics 1983; 71: 473-482

24. Hallman M, Merritt TA, Jarvenpaa AL, Boynton B, Mannino F, Gluck L, Moore T, Edwards D. Exogenous surfactant for treatment of severe respiratory distress syndrome: a randomized prospective clinical trial. J Pediatr 1985; 106: 963-969

25. Hein T, Lachmann B, Armbruster S, Smit JM, Voelkel N, Erdmann W. Pulmonary surfactant inhibits the cardiovascular effects of platelet activating factor (PAF), 5-hydroxytryptamine (5-HT) and angiotensin II. Am Rev Resp Dis 1987; 135: A506

26. Heldt GP, Pesonen E, Merritt TA, Elias W, Sahn DJ. Dynamic lung compliance, closure of the patent ductus arteriosus and surfactant therapy. Pediatr Res 1988; 23: 510A
Also published: Heldt GP, Pesonen E, Merritt TA, Elias W, Sahn DJ. Closure of the ductus

arteriosus and mechanics of breathing in preterm infants after surfactant replacement therapy. Pediatr Res 1989; 25: 305-310

27. Horbar JD, Sutherland J, Philip AGS, Little G, Vidyasagar D, Jobe AH, Mullett MD. Multicenter trial of single dose Surfactant-TA for treatment of respiratory distress syndrome. Pediatr Res 1988a; 23: 410A

    Also published: Horbar JD, Soll RF, Sutherland JM, Kotagal U, Philip AG, Kessler DL, Little GA, Edwards WH, Vidyasagar D, Raju TN, et al. A multicenter randomized, placebo-controlled trial of surfactant therapy for respiratory distress syndrome. N Engl J Med 1989; 320: 959-965

28. Horbar JD, Linderkamp O, Schachinger H, Versmold H, Duc G, Lemburg P, von Loewenich V, Minoli I, Riegel K. European trial of single dose surfactant-TA for treatment of respiratory distress syndrome. Pediatr Res 1988b; 23: 510A

29. Huber G, Mullane J, LaForce FM. The role of alveolar lining material in antibacterial defenses of the lung. Bull Europ Physiopath Resp 1976; 12: 178-179

30. Jarstrand C. Role of surfactant in the pulmonary defence system. In: Robertson B, van Golde LMG, Batenburg JJ (eds) Pulmonary Surfactant. Amsterdam, Elsevier 1984: 187-201

31. Jobe A, Ikegami M. The prematurely delivered lamb as a model for studies of neonatal adaptation. In: Nathanielsz PW (ed) Animal models in fetal medicine. Ithaca, Perinatology Press 1984: 1-30

32. Kendig JW, Notter RH, Cox C, Aschner JL, Benn S, Bernstein RM, Hendricks-Munoz K, Maniscalco WM, Metlay LA, Phelps DL, Sinkin RA, Wood BP, Shapiro DL. Surfactant replacement at birth: final analysis of a clinical trial and comparisons with similar trials. Pediatrics 1988; 82: 756-762

33. Kobayashi T, Kataoka H, Ueda T, Murakami S, Takada Y, Kokubo M. Effects of surfactant supplement and end-expiratory pressure in lung-lavaged rabbits. J Appl Physiol 1984; 57: 995-1001

34. Konishi M, Fujiwara T, Naito T, Takeuchi Y, Ogawa Y, Inukai K, Fujimura M, Nakamura H, Hashimoto T. Surfactant replacement therapy in neonatal respiratory distress syndrome: a multi-centre, randomized clinical trial: comparison of high- versus low-dose of Surfactant TA. Eur J Pediatr 1988a; 147: 20-25

35. Konishi M, Fujiwara T, Chida S, Takeuchi Y, Nakamura H, Ogawa Y, Okuyama K, Sibata T. Response to a single dose of exogenous surfactant: a multicenter study. In: Ross Laboratories special conference: Hot topics '88 in Neonatology 1988b: 48-61

36. Kwong MS, Egan EA, Notter RH, Shapiro DL. Double-blind clinical trial of calf lung surfactant extract for the prevention of hyaline membrane disease in extremely premature infants. Pediatrics 1985; 76: 585-592

37. Kwong MS, Egan EA. Routine use of Infasurf (calf lung surfactant extract) at birth for prematures ≤ 32 wks gestation. Pediatr Res 1988; 23: 415A

38. Lachmann B, Fujiwara T, Chida S, Morita T, Konishi M, Nakamura K, Maeta H. Surfactant replacement therapy in the experimental adult respiratory distress syndrome (ARDS). In: Cosmi EV,

Scarpelli EM (eds) Pulmonary Surfactant System. Amsterdam, Elsevier 1983: 231-235

39. Lachmann B. Possible function of bronchial surfactant. Eur J. Respir Dis 1985; 67: 49-61

40. Lachmann B, Becher G. Protective effect of lung surfactant on allergic bronchial constriction in guinea pigs. Am Rev Respir Dis 1986; 133: A118

41. Lachmann B, Bergmann KC. Surfactant replacement improves thorax-lung compliance and survival rate in mice with influenza infection. Am Rev Respir Dis 1987a; 135: A6

42. Lachmann B, Saugstad OD, Erdmann W. Effect of surfactant replacement on respiratory failure induced by free oxygen radicals. In: Schlag G, Redl H (eds) First Vienna Shock Forum, Part B: Monitoring and Treatment of Shock. New York, Alan Liss 1987b: 305-313

43. Lachmann B, Hallman M, Bergmann KC. Respiratory failure following anti-lung serum: study on mechanisms associated with surfactant system damage. Exp Lung Res 1987c; 12: 163-180

44. Lachmann B. Surfactant replacement in acute respiratory failure: animal studies and first clinical trials. In: Lachmann B (ed) Surfactant Replacement Therapy in Neonatal and Adult Respiratory Distress Syndrome. Berlin, Heidelberg, Springer Verlag 1988: 212-223

45. Lang MJ, Rhodes PG, Reddy S, Kurth CG, Merritt TA, Hall RT. Limitation of the effective use of human surfactant in established respiratory distress syndrome. Pediatr Res 1988; 23: 513A

46. Macklem PT, Proctor DF, Hogg JC. The stability of peripheral airways. Resp Physiol 1970; 8: 191-203

47. Matalon S, Holm BA, Notter RH. Mitigation of pulmonary hyperoxic injury by administration of exogenous surfactant. J Appl Physiol 1987; 62: 756-761

48. McCord FB, Curstedt T, Halliday HL, McClure G, Reid MMcC, Robertson B. Surfactant treatment and incidence of intraventricular haemorrhage in severe respiratory distress syndrome. Arch Dis Child 1988a; 63: 10-16

49. McCord B, Halliday HL, McClure G, Reid MMcC. Changes in pulmonary and cerebral blood flow after surfactant treatment for severe respiratory distress syndrome. In: Lachmann B (ed) Surfactant Replacement Therapy in Neonatal and Adult Respiratory Distress Syndrome. Berlin, Heidelberg, Springer-Verlag 1988b: 195-200

50. Merritt TA, Cochrane CG, Hallman M, Holcomb KE, Strayer D, Mannino F, Edwards DK, Gluck L. Reduction of lung injury by human surfactant treatment in respiratory distress syndrome. Chest 1983; 5: 27S-31S

51. Merritt TA, Hallman M, Bloom BT, Berry C, Benirschke K, Sahn D, Key T, Edwards D, Jarvenpaa AL, Pohjavuori M, Kankaanpaa K, Kunnas M, Paatero H, Rapola J, Jaaskelainen J. Prophylactic treatment of very premature infants with human surfactant. N Engl J Med 1986; 315: 785-790

52. Merritt TA, Hallman M. Surfactant replacement: a new era with many challenges for neonatal medicine. AJDC 1988a; 142: 1333-1339

53. Merritt TA, Hallman M. Human surfactant treatment of respiratory distress syndrome: recent experiences in prophylactic versus rescue treatment and an analysis of the role of SP-A in surfactant

fuction. In: Ross Laboratories special conference: Hot topics '88 in Neonatology 1988b: 82-85

54. Morley CJ, Bangham AD, Miller N, Davis JA. Dry artificial surfactant and its effect on very premature babies. Lancet 1981; 1: 64-68

55. Morley CJ, et al (Ten centre study group). Ten centre trial of artificial surfactant (artificial lung expanding compound) in very premature babies. Br Med J 1987; 294: 991-996

56. Morley CJ. Artificial surfactant: prophylaxis for respiratory distress syndrome. In: Lachmann B (ed) Surfactant Replacement Therapy in Neonatal and Adult Respiratory Distress Syndrome. Berlin, Heidelberg, Springer-Verlag 1988: 158-167

57. Mortensson W, Noack G, Curstedt T, Herin P, Robertson B. Radiologic observations in severe neonatal respiratory distress syndrome treated with the isolated phospholipid fraction of natural surfactant. Acta Radiol 1987; 28: 389-394

58. Noack G, Berggren P, Curstedt T, Grossmann G, Herin P, Mortensson W, Nilsson R, Robertson B. Severe neonatal respiratory distress syndrome treated with the isolated phospholipid fraction of natural surfactant. Acta Paediatr Scand 1987; 76: 697-705

59. Obladen M, Stevens P, Kattner E. Rapid response of oxygenation, slow response of compliance after liposomal phospholipid substitution in respiratory distress syndrome. In: Lachmann B (ed) Surfactant Replacement Therapy in Neonatal and Adult Respiratory Distress Syndrome. Berlin, Heidelberg, Springer-Verlag 1988: 168-180

60. Pattle RE. Properties, function, and origin of the alveolar lining layer. Nature 1955; 175: 1125-1126

61. Phibbs RH. A preliminary report of initial trial of Exosurf, a synthetic surfactant for the prevention and early treatment of hyaline membrane disease. In: Ross Laboratories special conference: Hot topics '88 in Neonatology 1988: 202-208

62. Raju TNK, Vidyasagar D, Bhat R, Sobel D, McCulloch KM, Anderson M, Macta H, Levy PS. Double-blind controlled trial of single-dose treatment with bovine surfactant in severe hyaline membrane disease. Lancet 1987; 1: 651-656

63. Reifenrath R. Surfactant action in bronchial mucus. In: Scarpelli EM (ed) Pulmonary Surfactant System. Amsterdam, Elsevier 1983: 339-347

64. Robertson B, et al (Collaborative European Multicenter Group). Surfactant replacement therapy for severe neonatal respiratory distress syndrome: an international randomized clinical trial. Pediatrics 1988a; 82: 683-691

65. Robertson B. The Curosurf experience in Europe. In: Ross Laboratories special conference: Hot topics '88 in Neonatology 1988b: 62-72

66. Robillard E, Alarie Y, Dagenais-Perusse P, Baril E, Guibeault A. Microaerosol administration of ß-y-Dipalmitoyl-L-α-Lecithin in the respiratory distress syndrome: a preliminary report. Canad Med Ass J 1964; 90: 55-57

67. Shapiro DL, Notter RH, Morin FC, Deluga KS, Golub LM, Sinkin RA, Weiss KI, Cox C. Double-blind, randomized trial of a calf lung surfactant extract administered at birth to very premature

infants for prevention of respiratory distress syndrome. Pediatrics 1985; 76: 593-599

68. Shapiro DL, Kendig JW, Notter RH, Reubens L, Cox C, Risemberg H, Bartoletti A, Dweck H. A multicenter randomized trial of pre-ventilatory versus post-ventilatory administration of surfactant (calf lung surfactant extract). In: Ross Laboratories special conference: Hot topics '88 in Neonatology 1988: 105-108

69. Shennan AT, Dunn MS, Possmayer F. CLSE experience in Canada. In: Ross Laboratories special conference: Hot topics '88 in Neonatology 1988: 116-142

70. Smyth JA, Metcalfe IL, Duffty P, Enhorning GE, Possmayer F, Olley PM, Bryan MH. Surfactant therapy in hyaline membrane disease. Pediatr Res 1981; 15: 681A

71. Smyth JA, Metcalfe IL, Duffty P, Possmayer F, Bryan MH, Enhorning G. Hyaline membrane disease treated with bovine surfactant. Pediatrics 1983; 71: 913-917

72. Soll RF, Hoekstra R, Fangman J, Corbet A, Adams J, James LS, Schulze K, Oh W, Roberts JD. Multicenter trial of single dose surfactant TA for prevention of respiratory distress syndrome. Pediatr Res 1988; 23: 425A

Also published: Soll RF, Hoekstra RE, Fangman JJ, Corbet AJ, Adams JM, James LS, Schulze K, Oh W, Roberts JD Jr, Dorst JP, et al. Multicenter trial of single-dose modified bovine surfactant extract (Survanta) for prevention of respiratory distress syndrome. Pediatrics 1990; 85: 1092-1102

73. Speer CP, Harms K, Muller U, Schroter W, Curstedt T, Robertson B. Treatment of severe respiratory distress syndrome in the premature infant with natural surfactant. Monatsschr Kinderheilkd 1988; 136: 65-70

74. Svenningsen N, Robertson B, Andreason B, Berggren P, Johnson B, Lindroth M. Endotracheal administration of surfactant in very low birth weight infants with respiratory distress syndrome. Crit Care Med 1987; 15: 918-922

75. Taeusch WH, Keough KMW, Williams M. Characterization of bovine surfactant for infants with respiratory distress syndrome. Pediatrics 1986; 77: 572-581

76. Van Golde LMG, Batenburg JJ, Robertson B. The pulmonary surfactant system. Biochemical aspects and functional significance. Physiol Rev 1988; 68: 374-455

77. Von Neergaard K. Neue Auffassungen über einen Grund-begriff der Atemmechanik. Z Ges Exp Med 1929; 66: 373-394

78. Whitsett JA, Ohning BL, Ross G, Meuth J, Weaver T, Holm BA, Shapiro DL, Notter RH. Hydrophobic surfactant-associated protein in whole lung surfactant and its importance for biophysical activity in lung surfactant extracts used for replacement therapy. Pediatr Res 1986; 20: 460-467

79. Wilkinson A, Jenkins PA, Jeffrey JA. Two controlled trials of dry artificial surfactant: early effects and later outcome in babies with surfactant deficiency. Lancet 1985; 2: 287-291

80. Wood BP, Sinkin RA, Kendig JW, Notter RH, Shapiro DL. Exogenous lung surfactant: effect on radiographic appearance in premature infants. Radiology 1987; 165; 11-13

CHAPTER 2

PULMONARY SURFACTANT FUNCTION IN ACUTE RESPIRATORY FAILURE

E.P. Eijking

INTRODUCTION

The adult respiratory distress syndrome (ARDS) was first described in 1967 by Ashbaugh and colleagues (1). Hopewell and Murray described a large number of disorders, including shock of any etiology, sepsis, pneumonia, aspiration of gastric contents, trauma, acute pancreatitis, drug overdose and many others which can lead to this pathological entity (41). ARDS is characterized by acute respiratory failure (ARF). An operational definition of ARDS includes at least the following features: an appropriate risk factor; tachypnea; dyspnea; severe hypoxemia refractory to increased inspiratory oxygen concentrations (PaO_2/FiO_2 < 150 mmHg); decreased lung compliance (total compliance of the respiratory system < 0.7 ml/cmH_2O/kg); bilateral diffuse infiltration on the chest radiograph, severe pulmonary edema and absence of left ventricular failure (pulmonary wedge pressure <12 - 18 mmHg) (22,23).

Despite increased sophistication in methods of respiratory and metabolic support, since its description in 1967, mortality associated with ARDS currently is still around 50-70% (21) whatever the criteria of diagnosis (76,102).

PATHOPHYSIOLOGY OF ACUTE RESPIRATORY FAILURE

Humoral and cellular mechanisms

In the pathogenesis of ARF/ARDS numerous cellular and humeral mechanisms are involved, including neutrophils, macrophages, platelets, complement-, kallikrein-kinin, coagulation and fibrinolytic systems. These mechanisms are functionally linked to each other, so that the activation of one system leads to the activation of the others. This results in synthesis of arachidonic acid metabolites in lung tissue, and generation and liberation of mediators from target cells. These mediators, such as proteases, arachidonic acid metabolites, oxygen radicals and others, directly damage cellular and tissue structure and attract more mediator-generating cells (82). As a result of damage to the alveolar-capillary membrane, high-permeability pulmonary edema, containing plasma-derived proteins, accumulates in the alveolar space.

Besides these complex mechanisms causing damage to the alveolar-capillary membrane, direct damage to lung tissue can be a result of noxes entering the airways,

such as aspiration of gastric contents, inhalation of toxic gases, micro-organisms entering the airways, and in near-drowning.

Role of surfactant

Bronchoalveolar lavage (BAL) material of ARDS patients demonstrates impaired surfactant function. Ashbaugh and colleagues found decreased lung compliance and increased minimal surface tension in lung extracts from two ARDS patients (1). Petty and coworkers examined the lungs from five patients who died from ARDS and found reduced lung compliance compared to normal lungs and the BAL fluid was found to have increased surface film compressibility (87). Von Wichert and Kohl found increased total phospholipid content and decreased dipalmitoyllecithin in lung homogenates from ARDS patients (113). Earlier, Lachmann and colleagues demonstrated a significant correlation between the duration of artificial ventilation and surfactant activity in lung homogenates from 16 ARDS patients (62). In BAL fluid from ARDS patients, Hallman and colleagues found a normal total phospholipid content, but a low lecithin/sphingomyelin (L/S) ratio, low concentrations of phosphatidylglycerol (PG) and disaturated lecithin, whereas concentrations of sphingomyelin and phosphatidylserine (PS) were high (31). Pison and colleagues found normal total phospholipid content in BAL fluid from ARDS patients, but a relative decrease in phosphatidylcholine (PC) and PG, and an increase in phosphatidylinositol (PI), phosphatidylethanolamine (PE) and sphingomyelin (88). Gregory and colleagues observed a decrease in total phospholipids and minimal surface tension in BAL fluid from ARDS patients and patients at-risk to develop ARDS (29). Also, in this study, in BAL fluid from ARDS patients decreased levels of PC, PG and increased levels of PI, PE and sphyngomyelin were observed; concentrations of surfactant-associated proteins SP-A and SP-B were also decreased. In BAL fluid from patients at-risk to develop ARDS, similar phospolipid profiles as in BAL fluid from ARDS patients were observed; also, there was a significant decrease in lavagable concentration of SP-A in BAL fluid of at-risk patients (29).

Due to increased permeability of the alveolar-capillary membrane in ARDS plasma proteins enter the alveolar space from the pulmonary circulation. Plasma proteins (such as fibrin(ogen), albumin, globulin and transferrin), hemoglobin and cell membrane lipids are known to inhibit pulmonary surfactant function in a dose-dependent way

(10,19,24,31,36-38,40,44-47,56,95,100,101). The existence of a specific plasma-derived surfactant inhibitor (MW=110.000 Da, unreduced) has been proposed (46).

Furthermore, due to damage to the lung, especially damage to surfactant-producing type II cells in ARDS, surfactant synthesis is decreased (4,34,39,94,107). This leads to a further decrease of surfactant concentration at the air-liquid interface. Due to diminished surfactant function, surface tension on the alveolar walls increases, leading to increased suction force across the alveolar-capillary membrane, with further accumulation of edema fluid in the alveolar spaces. Altogether, this leads to a vicious circle (73).

SURFACTANT TREATMENT IN ARF

A simple and rational hypothesis for treatment of ARF in which the surfactant system appears to be involved has been developed (72):

- If surfactant is necessary for keeping retractive forces as low as possible and for optimal gas exchange, any disturbance in the surfactant system will result in abnormalities in lung distensibility and gas exchange.
- If this is correct, surfactant replacement will restore lung distensibility and gas exchange to normal.

This hypothesis has been investigated in the last 10 years in different animal models of ARF and in a few patients with ARDS. The following section discusses selected animal models of ARF, with special reference to the models used in our laboratories to investigate disturbances of the surfactant system and/or surfactant replacement therapy.

ANIMAL MODELS

I-In vivo lung lavage: Experiments on postmortem lung specimens have revealed that a considerable portion of the alveolar phospholipids can be recovered by repeated washing of the airspaces (25). With this knowledge a model for ARF was developed by Lachmann and colleagues for use in different animals, in which pulmonary surfactant is removed

by repeated bi-lateral whole lung lavage with warm saline (64,67,96).

In these lavage models, severe respiratory insufficiency is defined as a decrease in PaO_2 to below 80 mmHg during artificial ventilation with 100% oxygen. The first lavage already results in significant reduction of thorax-lung compliance and repeated lavage results in a derangement of lung function persisting for at least 8 h. These disturbances, which include a 35% decrease in functional residual capacity (FRC), are almost certainly secondary to increased surface tension in the alveolar lining (42,48,64,67), especially as lung lavage with saline does not alter the elastic properties of the pulmonary parenchyma (42). However, removal of alveolar surfactant by in vivo lung lavage significantly increases permeability of the alveolo-capillary membrane, as demonstrated by Wollmer and coworkers in their studies on ^{99m}Tc-DTPA clearance (20). Histologic examination of lungs from animals 5 min after the last lavage shows atelectasis, desquamation of bronchial and bronchiolar epithelium, and incipient formation of hyaline membranes (64). Electron microscopic studies of the same lungs revealed necrosis and desquamation of alveolar type I cells. Some type II cells were also necrotic, but the majority of these cells seemed to be intact and should thus be available for synthesis and discharge of surfactant phospholipids (64).

Intratracheal instillation of exogenous surfactant in lavaged lungs results in dramatic improvement of gas exchange, even when the treatment is given 2 h after the lavage procedure (5,53,64,68). Improved blood gases are stable for at least 5 h (68), whereas in lavaged animals receiving no surfactant PaO_2 remains low despite ventilation with positive end-expiratory pressure (PEEP) and pure oxygen. Histologic sections from surfactant-treated animals showed a uniform pattern of well-aerated alveoli with only minimal intra-alveolar edema and hyaline membranes, whereas lavaged animals receiving no surfactant, ventilated with the same ventilator settings, had extensive atelectasis and prominent hyaline membranes (5,68).

The lung lavage models are useful for a variety of experimental purposes, including testing of different surfactant preparations (5,53,96), evaluation of pharmacologic agents stimulating discharge of alveolar phospholipids (63), and studies on the significance of different ventilator settings (7,67).

Recently the lavage model was used to investigate the influence of anti-surfactant monoclonal antibodies on the ability of exogenous surfactant to restore gas exchange (17)

(see Chapter 8). Also, the influence of plasma and edema fluid on exogenous surfactant function was tested using this model (see Chapters 3 and 4).

II-Pulmonary infection: Pneumonia continues to be a leading cause of death and ARDS is often associated with or complicated by pneumonia of different microbial etiology. Pulmonary infection causes direct injury to the alveolar membrane with destruction of both type I and type II cells (107). This results in permeability edema with decreased pulmonary compliance, decreased FRC, impaired gas exchange and decreased surfactant activity (33,79,105,110). Acute pneumonia-associated decrease of pulmonary function can be explained by decreased production of surfactant due to damage to type II cells, impairment of surfactant function by inhibitors present in edema fluid, increased breakdown of surfactant phospholipids and proteins during the inflammatory reaction to pulmonary infection, and increased loss of surfactant into the blood or into the airways.

We investigated the possibility of surfactant replacement therapy during respiratory failure due to pulmonary infection in three animal models with pneumonia of different microbial etiology.

IIa-Pneumocystis carinii: Pneumocystis carinii pneumonia (PCP) frequently occurs in immunocompromised patients and is currently the most frequent cause of pneumonia in patients suffering from the acquired immunodeficiency syndrome (AIDS) (90). Pneumocystis carinii infection leads to degenerative changes in type I pneumocytes with resulting increased permeability of the alveolar-capillary membrane and pulmonary edema (115). Furthermore, it was demonstrated in a PCP rat model with decreased pulmonary compliance, that pulmonary phospholipid content of BAL fluid was significantly decreased, whereas phospholipase A_2 was significantly increased (51,103). BAL fluid from patients suffering from PCP showed a decrease in total phospholipid content, a decrease in PC and PG, an increase in sphingomyelin, and an increase in total proteins (89).

We demonstrated in rats with PCP, suffering from severe respiratory failure, that instillation of a natural surfactant could significantly improve gas exchange (16) (see Chapter 7). Histologic examination clearly showed that lungs of untreated animals were filled with characteristic foamy edema, whereas lungs of surfactant treated animals were

clearly more aerated compared with saline-treated animals. Lung homogenates of all PCP animals showed large amounts of cysts, confirming the presence of PCP in all animals, whereas bacterial pneumonia was excluded in all animals.

IIb-Influenza A virus: Earlier, we reported the positive therapeutic effect of surfactant replacement on lung mechanics in mice with influenza A pneumonia (70). In this model, mice are exposed to a live nebulized influenza A virus and subsequently develop a severe pneumonia with lethal outcome within six days.

Recently (13) we showed that during the development of lethal influenza A pneumonia in mice, thorax-lung compliance (C/kg) and lung volume at PEEP=5 cmH_2O (V_5/kg) significantly decreased, whereas lung water content significantly increased. Furthermore, surface tension of BAL fluid significantly increased in infected animals, indicating loss of surfactant function. Surfactant replacement therapy (200 mg/kg BW) during the endstage of pneumonia increased C/kg and V_5/kg. Instillation of the vehicle for surfactant (1:2 mixture of H_2O and saline) in control animals did not significantly affect pulmonary compliance but significantly decreased V_5/kg.

IIc-Sendai virus: To study the effects of surfactant replacement therapy on gas exchange we developed a model of viral pneumonia, caused by Sendai virus infection of rats, closely resembling ARDS (14). In this model, rats are exposed to nebulized concentrated live Sendai virus. Subsequently, the animals develop a severe pneumonia with lethal outcome within four days. During this period gas exchange and pulmonary compliance significantly deteriorate whereas total protein content of BAL fluid significantly increases.

In another study using the same model (12), animals showed severely impaired gas exchange and acidosis 48 h after infection. Arterial oxygenation could not be sufficiently improved by artificial ventilation with high peak airway pressures and PEEP. At this time, at the same ventilator settings, surfactant replacement dramatically increased arterial oxygenation within 5 min and resulted in a fourfold increase in PaO_2 within 2 h (Fig. 1). Histological examination of surfactant-treated lungs showed clearly improved alveolar aeration compared to saline-treated controls.

Results from the influenza A virus pneumonia model in mice and those from the Sendai virus pneumonia model in rats lead us to conclude that intratracheal

administration of surfactant is a promising approach in the treatment of severe respiratory failure associated with viral pneumonia.

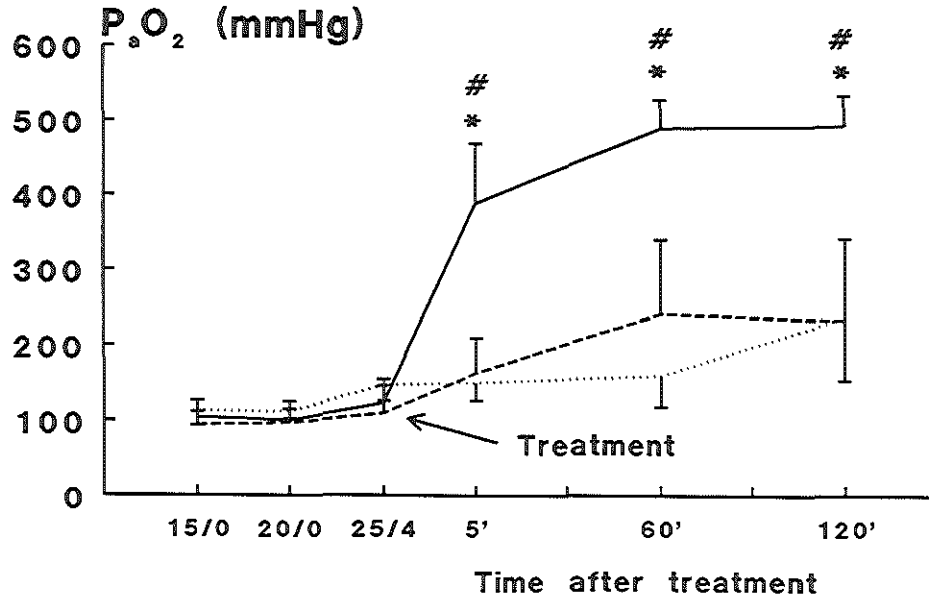


Figure 1. PaO_2 values (mean ± SD) 48 h after infection with a lethal dose of Sendai virus, during pressure-controlled artificial ventilation with pure oxygen, and after treatment with surfactant (solid line, n=9), saline (broken line, n=7) or respiratory support only (dotted line, n=6); *p<0.05, surfactant-treated animals vs. saline-treated controls; #p<0.05, vs. pre-treatment values at the same ventilator settings; 15/0 = peak airway pressure (P_{peak}) = 15 cmH_2O; 20/0 = P_{peak} = 20 cmH_2O; 25/4 = P_{peak} = 25 cmH_2O with PEEP = 4 cmH_2O (from Van Daal et al [12]).

III-Hydrochloric acid instillation: The pulmonary effects of acid aspiration have been extensively documented in animal models (2,28,111). Data from these studies show that in the pH range below 2.5, the degree of lung injury is proportional to the hydrogen ion concentration of the aspirated material (for review see [26]). Hydrochloric acid (HCl) aspiration leads to damage to the alveolar-capillary membrane with subsequent increased permeability to and accumulation of plasma-derived proteins. It is also likely that

surfactant is directly damaged by HCl. Shortly after acid instillation, type II pneumocytes show cytoplasmic swelling (28) and the surface properties of alveolar lavage fluid and lung extracts are characterized by increased minimal surface tension and decreased hysteresis (3,8).

Several studies have been performed on animals suffering from respiratory failure due to HCl aspiration and treated with surfactant. Kobayashi and colleagues demonstrated that surfactant instillation could only partly restore gas exchange in rabbits suffering from respiratory failure due to HCl aspiration after intra-alveolar lung edema was removed by BAL; surfactant, when given without prior lung lavage, only prevented further deterioration of blood gases (54). Lamm and coworkers showed improved lung distensibility in rabbits receiving surfactant 5 min after HCl aspiration, but no effect was seen on blood gases (74). Recently we showed that intratracheal surfactant instillation within 10 min after HCl aspiration could prevent development of respiratory failure, whereas surfactant, given after deterioration of gas exchange, had no effect on pulmonary gas exchange (18) (see Chapter 5). In another study we demonstrated that lung function could be restored in rats suffering from respiratory failure due to HCl aspiration after lavaging the lungs with a diluted surfactant suspension, or by lavaging the lungs with saline, followed by surfactant treatment (104) (see Chapter 6).

Because surfactant treatment alone does not suffice to improve pulmonary function it is speculated that BAL with saline (followed by surfactant treatment), or with a diluted surfactant suspension, removes the inhibitory proteins present in the alveolar edema fluid, resulting in improved efficacy of surfactant treatment.

IVa-Immunological mechanisms: Monoclonal antibodies: Inactivation of the small (≤ 15kDa) hydrophobic surfactant proteins decreases surfactant function with subsequent loss of pulmonary function, resulting in ARDS. Suzuki and coworkers (109) intraperitoneally inoculated mice with hybridoma cells, producing a monoclonal antibody against the 15 kDa surfactant protein. Subsequently, animals developed respiratory failure within 9 days with markedly reduced thorax-lung compliance, lung congestion, alveolar collapse, hemorrhagic pulmonary edema and hyaline membranes, mimicking ARDS. Recently, we also demonstrated the importance of this surfactant-associated protein for surfactant function in a surfactant replacement study using the lavage model

in rats. It was shown that inhibition of an 11 kDa surfactant protein caused loss of effectiveness of the used surfactant preparation (17) (see Chapter 8). Kobayashi and coworkers demonstrated that as a result of inactivation of surfactant by a monoclonal anti-surfactant antibody directed against SP-B, this preparation was not able to improve lung compliance in immature rabbit fetuses (55). In another study Robertson and colleagues demonstrated that intratracheal instillation of a monoclonal antibody against SP-B in near-term newborn rabbits caused a decrease in thorax-lung compliance, when compared to rabbits which received nonspecific IgG (97). These studies demonstrate that SP-B is important for surfactant function and inhibition of this protein in a surfactant preparation makes the preparation inactive.

IVb-Immunological mechanisms: Anti-lung serum: The structural and functional pulmonary abnormalities due to capillary leakage in ARDS can be imitated in experimental animals by administration of anti-lung serum (59-61). This serum contains antibodies against surfactant-associated proteins and against the basement membrane of alveolar capillaries.

Earlier, Lachmann and colleagues demonstrated in artificially ventilated guinea pigs, that intravenous injection of anti-lung serum leads to respiratory failure with decrease of lung compliance, hypoxemia, and respiratory and metabolic acidosis. Morphologic lesions include interstitial and intra-alveolar edema, hemorrhage, and desquamation of alveolar epithelium. These structural abnormalities are associated with a marked reduction in the phospholipid content of the lungs (71). The decrease in lung compliance reflects accumulation of fluids in the air spaces and inactivation of surfactant components by plasma proteins. Instillation of a single dose of exogenous natural surfactant (280-350 mg/kg BW) after deterioration of blood gases improved gas exchange.

V-Other models for ARF and surfactant treatment have been published (Table 1). These will not be discussed in this chapter. Table 1 does not contain all literature references; for more detailed information, see Robertson and Lachmann (96).

Table 1: Animal models

Model		Animals	Source
Immature newborn animals		Rabbits	B. Lachmann (65,66) R. Nilsson (84) B. Robertson (96)
		Lambs	M. Ikegami (43,44)
Neurogenic ARDS	Head injury	Cats	D.J. Crittenden (11)
	Vagotomy	Rats	V.E. Goldenberg (27) D. Berry (6)
Oxygen toxicity		Different species	J. Klein (52) C.G. Cochrane (9) J.J. Ennema (19)
Oxydant producing enzymes	Xanthine oxidase	Guinea pigs	O.D. Saugstadt (99) B. Lachmann (69)
Septicemia	E. coli/E. coli endotoxin	Rats	K.T. Oldham (86) J.E. Rinaldo (92)
	Pseudomonas aeruginosa	Pigs Dogs	R.A. Mustard (81) P. Hanly (33)
N-nitroso-N-methylurethrane (NNNMU)		Rats Rabbits Dogs	J.D. Harris (34) J.F. Lewis (75) S.F. Ryan (98)
Oleic acid		Rabbits Sheep	S.B. Hall (30) M. Zelter (116)
Paraquat		Mice Rats	B.W. Manktelow (77) B. Robertson (93)
Hemorrhagic shock		Dogs Baboons	J.N. Henry (35) G.S. Moss (80)
Tumor necrosis factor (TNF)		Guinea pigs	K.E. Stephens (106)
BAL with plasma		Dogs	G.F. Nieman (83)
Bile acid aspiration		Rabbits	T. Kaneko (50)

CASE REPORTS

A few reports on ARDS patients with different etiologies treated with surfactant have been published (Table 2: see Appendix B). The surfactant preparations used varied from synthetic to natural surfactants. Dosage used to treat this syndrome varied from very low (<20 mg/kg body weight) to high (300 mg/kg body weight); the method of administration varied from single intratracheal instillation to continuous nebulization during 5 days. Although these patients treated with surfactant are "worst scenario cases" and most case reports are poorly documented, (transient or incomplete) restoration of respiratory failure was observed; no adverse immunological reactions were reported (Table 2: see Appendix B). Also, case studies have been reported during international congresses. For example, during a congress in Tokyo (organized by the Japanese Society for Anesthesia and Intensive Care, and Tokyo Tanabe, July 1992) a few reports on surfactant treatment in adults suffering from ARF were presented. In these patients a natural surfactant at a dosage of 240-480 mg in total was selectively instilled in atelectatic lung parts by means of a bronchofiberscope. Atelectasis in these patients was a result of, e.g. pneumothorax after cardiopulmonary resuscitation (CPR), postoperative intrapulmonary hemorrhage, postoperative pneumonia, or postoperative atelectasis. The results were not consistent, although some improvements in gas exchange, lung mechanics and chest X-rays were reported after one or more days. From these results it could be concluded that, due to the small amounts used, exogenous surfactant had no clear effect on lung function.

Since 1980 reports on surfactant replacement trials for treatment of neonatal RDS have been published (see Chapter 1, Appendix A), and surfactant treatment has become accepted in the neonatal intensive care unit. However, discussions still continue concerning the time of treatment (e.g. rescue vs. prophylactic), dosage of surfactant and type of mechanical ventilation to be used. Concerning the dosage of surfactant in RDS, it has been reported that some neonates did not respond to, or had an only transient improvement after a single treatment of low-dose surfactant (32,57). It has been suggested that in these patients surfactant was gradually inhibited by proteins present in the pulmonary edema fluid. This hypothesis was confirmed in other clinical studies in which better clinical outcome was seen in neonates treated with either a higher dose of

surfactant (57) or with multiple doses (15).

For treatment of ARDS many questions remain unanswered: for example, what type and what dosage of surfactant should be used, when should surfactant treatment start, how should it be administered, etc. To address these questions controlled clinical trials need to be performed.

CONCLUDING REMARKS

All the experimental and clinical findings summarized above support the contention that ARF/ARDS is caused by multiple factors, leading to increased permeability of the alveolar-capillary membrane and disturbance of the pulmonary surfactant system. The wide variations of clinical ARDS are presented in case reports and simulated in the experimental models of ARF described above, which all differ with respect to etiology, severity of respiratory distress, and time course. In all experimental models in which surfactant replacement therapy has been evaluated, a significant beneficial effect of surfactant instillation was observed. Although much still remains unclear and much experimental work still has to be done, these findings provide additional indication of the potential therapeutic significance of surfactant replacement therapy in ARDS. In order to get a clear view on the therapeutic effects in patients with ARDS, controlled clinical trials should be performed, addressing questions such as which patient should be treated (e.g. in extended pulmonary fibrosis little or no effect can be expected after surfactant administration), the dosage and type of surfactant to be used, the method of administration, the type of ventilatory support, and many others.

REFERENCES

1. Ashbaugh DG, Bigelow DB, Petty TL, Levine BE. Acute respiratory distress in adults. Lancet 1967; 2: 319-323
2. Awe WD, Fletcher WS, Jacob SW. The pathophysiology of aspiration pneumonitis. Surgery 1966; 60: 232-239
3. Baum KF, Beckman DL. Aspiration pneumonitis and pulmonary phospholipids. J Trauma 1976; 16: 782-787
4. Bachofen M, Weibel ER. Structural alterations of lung parenchyma in the adult respiratory distress

syndrome. Clin Chest Med 1982; 3: 35-56

5. Berggren P, Lachmann B, Curstedt T, Grossmann G, Robertson B. Gas exchange and lung morphology after surfactant replacement in experimental adult respiratory distress syndrome induced by repeated lung lavage. Acta Anaesthesiol Scand 1986; 30: 321-328
6. Berry D, Ikegami M, Jobe A. Respiratory distress and surfactant inhibition following vagotomy in rabbits. J Appl Physiol 1986; 61: 1741-1748
7. Bos JAH, Schairer W, Schaffers JT, Tenbrinck R, TenHave-Opbroek AAW, Bakker WH, Wollmer P, Lachmann B. Effects of high frequency jet ventilation on the clearance of ^{99m}Tc-DTPA in respiratory failure in rabbits. Brit J Anaesth 1989; 63: 59S-64S
8. Brown ES. Aspiration and lung surfactant. Anesth Analg 1967; 46: 665-672
9. Cochrane CG, Spragg R, Revak SD. Pathogenesis of the adult respiratory distress syndrome: evidence of oxidant activity in bronchoalveolar lavage fluid. J Clin Invest 1983; 71: 754-761
10. 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. Biochemistry 1990; 29: 8424-8429
11. Crittenden DJ, Beckman DL. Traumatic head injury and pulmonary damage. J Trauma 1982; 22: 766-769
12. Van Daal GJ, So KL, Gommers D, Eijking EP, Fiévez RB, Sprenger MJ, Van Dam DW, Lachmann B. Intratracheal surfactant administration restores gas exchange in experimental adult respiratory distress syndrome associated with viral pneumonia. Anesth Analg 1991; 72: 589-595
13. Van Daal GJ, Bos JAH, Eijking EP, Gommers D, Hannappel E, Lachmann B. Surfactant replacement therapy improves pulmonary mechanics in end-stage influenza A pneumonia in mice. Am Rev Respir Dis 1992; 145: 859-863
14. Van Daal GJ, Eijking EP, So KL, Fiévez RBAM, Sprenger MJW, Van Dam DW, Erdmann W, Lachmann B. Acute respiratory failure during pneumonia induced by Sendai virus. Adv Exp Med Biol 1992; 316: 319-326
15. Dunn MS, Shennan AT, Possmayer F. Single- versus multiple-dose surfactant replacement therapy in neonates of 30 to 36 weeks' gestation with respiratory distress syndrome. Pediatrics 1990; 86: 564-571
16. Eijking EP, Van Daal GJ, Tenbrinck R, Luijendijk A, Sluiters JF, Hannappel E, Lachmann B. Effect of surfactant replacement on pneumocystis carinii pneumonia in rats. Intensive Care Med 1991; 17: 475-478
17. Eijking EP, Strayer DS, Van Daal GJ, Tenbrinck R, Merritt TA, Hannappel E, Lachmann B. In vivo and in vitro inactivation of bovine surfactant by an anti-surfactant monoclonal antibody. Eur Respir J 1991; 4: 1245-1250
18. Eijking EP, Gommers D, So KL, de Maat MPM, Mouton JW, Lachmann B. Prevention of respiratory failure after hydrochloric acid aspiration by intratracheal surfactant instillation in rats.

Anesth Analg 1993; 76: 472-477

19. Ennema JJ, Kobayashi T, Robertson B, Curstedt T. Inactivation of exogenous surfactant in experimental respiratory failure induced by hyperoxia. Acta Anaesthesiol Scand 1988; 32: 665-671
20. Evander E, Wollmer P, Jonson B, Lachmann B. Pulmonary clearance of ^{99m}Tc-DTPA: effects of surfactant depletion by lung lavage. J Appl Physiol 1987; 62: 1611-1614
21. Evans TW. Adult respiratory distress syndrome. Med Int 1989; 71: 2944-2947
22. Fein AM, Lippmann M, Holtzman H, Eliraz A, Goldberg SK. The risk factors, incidence, and prognosis of ARDS following septicemia. Chest 1983; 83: 40-42
23. Fowler AA, Hamman RF, Good JT, Benson KN, Baird M, Eberle DJ, Petty TL, Hyers TM. Adult respiratory distress syndrome: risk with common predispositions. Ann Intern Med 1983; 98: 593-597
24. Fuchimukai T, Fujiwara T, Takahashi A, Enhorning G. Artificial pulmonary surfactant inhibited by proteins. J Appl Physiol 1987; 62: 429-437
25. Fujiwara T, Adams FH, El-Salawy A, Sipos S. "Alveolar" and whole lung phospolipids of newborn lambs. Proc Soc Exp Biol Med (N.Y.) 1968; 127: 962-969
26. Gibbs CP, Modell JH. Management of aspiration pneumonitis. In: Miller RD (ed) Anesthesia. New York, Edinburgh, London, Melbourne: Churchill Livingstone, 1990: 1293-1319
27. Goldenberg VE, Buckingham S, Sommers SC. Pulmonary alveolar lesions in vagotomized rats. Lab Invest 1967; 16: 693-705
28. Greenfield LJ, Singleton RP, McCaffree DR, Coalson JJ. Pulmonary effects of experimental graded aspiration of hydrochloric acid. Ann Surg 1969; 170: 74-86
29. Gregory TJ, Longmore WJ, Moxley MA, Whitsett JA, Reed CR, Fowler AA, Hudson LD, Maunder RJ, Crim C, Hyers TM. Surfactant chemical composition and biophysical activity in acute respiratory distress syndrome. J Clin Invest 1991; 88: 1976-1981
30. Hall SB, Notter RH, Smith RJ, Hyde RW. Altered function of pulmonary surfactant in fatty acid lung injury. J Appl Physiol 1990; 69: 1143-1149
31. Hallman M, Spragg R, Harrell JH, Moser KM, Gluck L. Evidence of lung function abnormality in respiratory failure. Study of bronchoalveolar lavage phospholipids, surface activity phospholipase activity, and plasma myoinositol. J Clin Invest 1982; 70: 673-683
32. Hallman M, Merritt TA, Jarvenpaa A-L, Boynton B, Mannino F, Gluck L, Moore T, Edwards D. Exogenous human surfactant for treatment of severe respiratory distress syndrome: a randomized clinical trial. J Pediatrics 1985; 106: 963-969
33. Hanly P, Light RB. Lung mechanics, gas exchange, pulmonary perfusion, and hemodynamics in a canine model of acute Pseudomonas pneumonia. Lung 1987; 165: 305-322
34. Harris JD, Jackson F, Moxley MA, Longmore WJ. Effect of exogenous surfactant instillation on experimental acute lung injury. J Appl Physiol 1989; 66: 1846-1851
35. Henry JN, McArdle AH, Bounous G, Hampson LG, Scott HJ, Gurd FN. The effect of experimental hemorrhagic shock on pulmonary alveolar surfactant. J Trauma 1967; 7: 691-726

36. Holm BA, Notter RH, Finkelstein JN. Surface property changes from interactions of albumin with natural lung surfactant and extracted lung lipids. Chem Phys Lipids 1985; 38: 287-298
37. Holm BA, Notter RH. Effects of hemoglobin and cell membrane lipids on pulmonary surfactant activity. J Appl Physiol 1987; 63: 1434-1442
38. Holm BA, Enhorning G, Notter RH. A biophysical mechanism by which plasma proteins inhibit lung surfactant activity. Chem Phys Lipids 1988; 49: 49-55
39. Holm BA, Matalon S, Finkelstein JN, Notter RH. Type II pneumocyte changes during hyperoxic lung injury and recovery. J Appl Physiol 1988; 65: 2672-2678
40. Holm BA, Venkitaraman AR, Enhorning G, Notter RH. Biophysical inhibition of synthetic lung surfactants. Chem Phys Lipids 1990; 52: 243-250
41. Hopewell PC, Murray J. The adult respiratory distress syndrome. In: Shibel EM, Moser KM (eds) Respiratory emergencies. St Louis: CV Mosby, 1977: 101-102
42. Huber GL, Edmunds LH, Finley TN. Effects of experimental saline lavage on pulmonary mechanics and morphology. Am Rev Respir Dis 1971; 104: 337-347
43. Ikegami M, Adams FH, Towers B, Osher AB. The quantity of natural surfactant necessary to prevent the respiratory distress syndrome in premature lambs. Pediatr Res 1980; 14: 1082-1085
44. Ikegami M, Jobe A, Jacobs H, Jones S. Sequential treatments of premature lambs with an artificial surfactant and natural surfactant. J Clin Invest 1981; 68: 491-496
45. Ikegami M, Jacobs H, Jobe A. Surfactant function in respiratory distress syndrome. J Pediatr 1983; 102: 443-447
46. Ikegami M, Jobe A, Jacobs H, Lam R. A protein from airways of premature lambs that inhibits surfactant function. J Appl Physiol 1984; 57: 1134-1142
47. Ikegami M, Agata Y, Elkady T, Hallman M, Berry D, Jobe A. Comparison of four surfactants: in vitro surface properties and responses of preterm lambs to treatment at birth. Pediatrics 1987; 79: 38-46
48. Johnson JWC, Permutt S, Sipple JH, Salem ES. Effect of intra-alveolar fluid on pulmonary surface tension properties. J Appl Physiol 1964; 19: 769-777
49. Joka Th, Obertacke U. Neue medikamentöse Behandlung im ARDS: Effekt einer intrabronchialen xenogenen Surfactantapplikation. Z Herz-, Thorax-,Gefäßchir 1989; 3 (Suppl 1): 21-24
50. Kaneko T, Sato T, Katsuya H, Miyauchi Y. Surfactant therapy for pulmonary edema due to intratracheally injected bile acid. Crit Care Med 1990; 18: 77-83
51. Kernbaum S, Masliah J, Alcindor LG, Bouton C, Christol D. Phospholipase activities of bronchoalveolar lavage fluid in rat Pneumocystis carinii pneumonia. Br J Exp Path 1983; 64: 75-80
52. Klein J. Normobaric pulmonary oxygen toxicity. Anesth Analg 1990; 70: 195-207
53. Kobayashi T, Kataoka H, Ueda T, Murakami S, Takada Y, Kokubo M. Effect of surfactant supplement and end-expiratory pressure in lung-lavaged rabbits. J Appl Physiol 1984; 57: 995-1001
54. Kobayashi T, Ganzuka M, Tanigushi J, Nitta K, Murakami S. Lung lavage and surfactant

replacement for hydrochloric acid aspiration in rabbits. Acta Anaesthesiol Scand 1990; 34: 216-221

55. Kobayashi T, Nitta K, Takahashi R, Kurashima K, Robertson B, Suzuki Y. Activity of pulmonary surfactant after blocking the associated proteins SP-A and SP-B. J Appl Physiol 1991; 71: 530-536

56. Kobayashi T, Nitta K, Ganzuka M, Inui S, Grossmann G, Robertson B. Inactivation of exogenous surfactant by pulmonary edema fluid. Pediatr Res 1991; 29: 353-356

57. Konishi M, Fujiwara T, Naito T, Takeuchi Y, Ogawa Y, Inukai K, Fujimura M, Nakamura H, Hashimoto T. Surfactant replacement therapy in neonatal respiratory distress syndrome: A multi-centre, randomized clinical trial: comparison of high- versus low-dose of Surfactant TA. Eur J Pediatr 1988; 147: 20-25

58. Kurashima K, Ogawa H, Ohka T, Fujimura M, Matsuda T, Kobayashi T. A pilot study of surfactant inhalation for the treatment of asthmatic attack. Jpn J Allergol 1991; 40: 160-163

59. Lachmann B, Bergmann K-Ch, Vogel J. Experimentelles Atemnotsyndrom nach Injektion von Anti-Lungenserum. I. Atemmechanische, roentgenologische und blutanalytische Befunde. Pädiat Grenzgeb 1973; 12: 347-364

60. Lachmann B, Bergmann K-Ch, Winsel K, Eckert H, Wuthe H, Vogel J. Experimentelles Atemnotsyndrom nach Injektion von Anti-Lungenserum. II. Morphologische, biochemische, physikochemische und immunologische Befunde. Pädiat Grenzgeb 1973; 12: 403-414

61. Lachmann B, Bergmann K-Ch, Winsel K, Müller E, Petro W, Schäfer C, Vogel J. Experimentelles Atemnotsyndrom nach Injektion von Anti-Lungserum. III. Atemmechanische, blutgasanalytische, physikochemische und biochemische Befunde im chronischen Versuch; Therapieversuche. Pädiat Grenzgeb 1975; 14: 211-233

62. Lachmann B, Bergmann K-C, Enders K, Friebel L, Gehlmann B, Grossmann G, Hoffmann D, Kuckelt W, Malmquist E, Robertson B, Seidel M, Vogel J, Winsel K. Können pathologische Veränderungen im Surfactant-System der Lunge zu einer akuten respiratorischen Insuffizienz bein Erwachsenen führen? In: Danzmann E (ed) Anaesthesia 77, Proceedings of the 6th Congress of the Society of Anaesthesiology and Rescuscitation of the G.D.R., Vol 1. Berlin, 1977: 337-353

63. Lachmann B, Robertson B, Gehlmann B, Enders K, Seidel M, Petro W, Vogel J. Experimental respiratory distress induced by removal of alveolar surfactant with bronchial lavage. In: Georgiev GA (ed) Lung Lipid Metabolism, Mechanisms of its Regulation and Alveolar Surfactant, Proceedings of an International Symposium, Varna, May 19-22, 1976. Publishing House of the Bulgarian Academy of Sciences, Sofia, 1978: 303-313

64. Lachmann B, Robertson B, Vogel J. In vivo lung lavage as an experimental model of the respiratory distress syndrome. Acta Anaesthesiol Scand 1980; 24: 231-236

65. Lachmann B, Grossmann G, Freyse J, Robertson B. Lung-thorax compliance in the artificially ventilated premature rabbit neonate in relation to variations in inspiration:expiration ratio. Pediatr Res 1981; 15: 833-838

66. Lachmann B, Berggren P, Curstedt T, Grossmann G, Robertson B. Combined effects of surfactant

substitution and prolongation of inspiration phase in artificially ventilated premature newborn rabbits. Pediatr Res 1982; 16: 921-927

67. Lachmann B, Jonson B, Lindroth M, Robertson B. Modes of artificial ventilation in severe respiratory distress syndrome. Lung function and morphology in rabbits after wash-out of alveolar surfactant. Crit Care Med 1982; 10: 724-732

68. Lachmann B, Fujiwara T, Chida S, Morita T, Konishi M, Nakamura K, Macta H. Surfactant replacement therapy in the experimental adult respiratory distress syndrome (ARDS). In: Cosmi EV, Scarpelli EM (eds.) Pulmonary Surfactant System. Amsterdam, Elsevier, 1983: 231-235

69. Lachmann B, Saugstad OD, Erdmann W. Effects of surfactant replacement on respiratory failure induced by free oxygen radicals. First Vienna Shock Forum, Part B: Monitoring and Treatment of Shock: 1987: 305-313

70. Lachmann B, Bergmann KC. Surfactant replacement improves thorax-lung compliance and survival rate in mice with influenza infection. Am Rev Respir Dis 1987; 135: A6

71. Lachmann B, Hallman M, Bergmann KC. Respiratory failure following anti-lung serum: study on mechanisms associated with surfactant system damage. Exp Lung Res 1987; 12: 163-180

72. Lachmann B. Surfactant replacement in acute respiratory failure: animal studies and first clinical trials. In: Lachmann B (ed) Surfactant replacement therapy in neonatal and adult respiratory distress syndrome. Berlin, Springer-Verlag, 1988: 212-223

73. Lachmann B. Open up the lung and keep the lung open. Intensive Care Med 1992; 18: 319-321

74. Lamm WJE, Albert RK. Surfactant replacement improves lung recoil in rabbit lungs after acid aspiration. Am Rev Respir Dis 1990; 142: 1279-1283

75. Lewis JF, Ikegami M, Jobe AH. Altered surfactant function and metabolism in rabbits with acute lung injury. J Appl Physiol 1990; 69: 2303-2310

76. Lyrene RK, Troug WE. Adult respiratory distress syndrome in a pediatric intensive care unit: predisposing conditions, clinical course, and outcome. Pediatrics 1981; 67: 790-795

77. Manktelow BW. The loss of pulmonary surfactant in paraquat poisoning. Brit J Exp Path 1967; 48: 366-369

78. Marraro G. Respiratory emergencies and supplementary surfactant in the treatment of severe RDS in leukaemic adolescents. In: Cosmi EV, Di Renzo GC, Anceschi MM (eds) The surfactant system of the lung: prevention and treatment of neonatal and adult respiratory distress syndrome. Macmillan Press, 1991: 198-206

79. Mink SN, Light RB, Wood LD. Effect of pneumococcal lobar pneumonia on canine lung mechanics. J Appl Physiol 1981; 50: 283-291

80. Moss GS, Newson B, Das Gupta TK. The normal histochemistry and the effect of hemorrhagic shock on the pulmonary surfactant system. Surg Gynaec Obstet 1975; 140: 53-58

81. Mustard RA, Fusher J, Hayman S, Matlow A, Mullen JBM, Odumeru J, Roomi MW, Schouten BD, Swanson HT. Cardiopulmonary responses to Pseudomonas septicemia in swine: An improved model

of the adult respiratory distress syndrome. Lab Anim Science 1989; 39: 37-43

82. Neuhof H. Actions and interactions of mediator systems and mediators in the pathogenesis of ARDS and multiorgan failure. Acta Anaesthesiol Scand 1991; 35 (Suppl 95): 7-14

83. Nieman GF, Goyette D, Paskanik A, Brendenberg C. Surfactant displacement by plasma lavage results in pulmonary edema. Surgery 1990; 107: 677-683

84. Nilsson R. The artificially ventilated preterm rabbit neonate as experimental model of hyaline membrane disease. Acta Anaesthesiol Scand 1982; 26: 89-103

85. Nosaka S, Sakai T, Yonekura M, Yoshikawa K. Surfactant for adults with respiratory failure. Lancet 1990; 1: 947-948

86. Oldham KT, Guice KS, Stetson PS, Wolfe RR. Bacteremia-induced suppression of alveolar surfactant production. J Surg Res 1989; 47: 397-402

87. Petty TL, Silvers GW, Paul GW, Stanford RE. Abnormalities in lung elastic properties and surfactant function in adult respiratory distress syndrome. Chest 1979; 75: 571-574

88. Pison U, Seeger W, Buchhorn R, Joka T, Brand M, Obertacke U, Neuhof H, Schmit-Neuerburg KP. Surfactant abnormalities in patients with respiratory failure after multiple trauma. Am Rev Respir Dis 1989; 140: 1033-1039

89. Prevost MC, Escamilla R, Hermant Ch, Carlven C, Krempf M. Permissive role of surfactant abnormalities for saprophyte-pathogen transformation of pneumocystis carinii in HIV infected patients. Am Rev Respir Dis 1992; 145: A245

90. Rankin JA, Collman R, Daniele RP. Acquired immune deficiency syndrome and the lung. Chest 1988; 94: 155-164

91. Richman PS, Spragg RG, Robertson B, Merritt TA, Curstedt T. The adult respiratory distress syndrome: first trials with surfactant replacement. Eur Respir J 1989; 2 (Suppl 3): 109s-111s

92. Rinaldo JE, Dauber JH, Christman J, Rogers RM. Neutrophil alveolitis following endotoxemia. Enhancement by previous exposure to hyperoxia. Am Rev Respir Dis 1984; 130: 1065-1071

93. Robertson B, Enhorning G, Ivemark B, Malmquist E, Modée J. Experimental respiratory distress induced by paraquat. J Path 1971; 103: 239-244

94. Robertson B. Paraquat poisoning as an experimental model of the idiopathic respiratory distress syndrome. Bull Physiopath Resp 1973; 1433-1452

95. Robertson B, Berry D, Curstedt T, Grossmann G, Ikegami M, Jacobs H, Jobe A, Jones. Leakage of protein in the immature rabbit lung; effect of surfactant replacement. Respir Physiol 1985; 61: 265-276

96. Robertson B, Lachmann B. Experimental evaluation of surfactants for replacement therapy. Exp Lung Res 1988; 14: 279-310

97. Robertson B, Kobayashi T, Ganzuka M, Grossmann G, Li WZ, Suzuki Y. Experimental neonatal respiratory failure induced by a monoclonal antibody to the hydrophobic surfactant-associated protein SP-B. Pediatr Res 1991; 30: 239-243

98. Ryan SF, Liau DF, Bell ALL, Hashim SA, Barrett CR. Correlation of lung compliance and quantities of surfactant phospolipids after acute alveolar injury from N-nitroso-N-methylurethane in the dog. Am Rev Respir Dis 1981; 123: 200-204

99. Saugstad OD, Hallman M, Abraham J, Cochrane GG, Epstein B, Gluck L. Hypoxanthine and oxygen induced lung injury: a possible basic mechanism of tissue damage? Pedriatr Res 1984; 18: 501-504

100. Seeger W, Stöhr G, Wolf HRD, Neuhof H. Alteration of surfactant function due to protein leakage: special interaction with fibrin monomer. J Appl Physiol 1985; 58: 326-338

101. Seeger W, Günther A, Thede C. Differential sensitivity to fibrinogen inhibition of SP-C vs. SP-B based surfactants. Am J Physiol 1992; 261: L286-L291

102. Shale DJ. The adult respiratory distress syndrome - 20 years on. Thorax 1987; 42: 642-645

103. Sheehan PM, Stokes DC, Yeh Y, Hughes WT. Surfactant phospholipids and lavage phospholipase A2 in experimental Pneumocystis carinii pneumonia. Am Rev Respir Dis 1986; 134: 526-531

104. So KL, Eijking EP, Gommers D, de Buijzer E, Beukenholdt RW, Lachmann B. Different surfactant treatment strategies for respiratory failure induced by hydrochloric acid aspiration in rats. Intensive Care Med 1992; 18 (Suppl 2): S111

105. Somerson NL, Kontras SB, Pollack JD, Weiss HS. Pulmonary compliance: alteration during infection. Science 1971; 171: 66-68

106. Stephens KE, Ishizaka A, Larrick JW, Raffin TA. Tumor necrosis factor causes increased pulmonary permeability and edema. Comparison to septic acute lung injury. Am Rev Respir Dis 1988; 137: 1364-1370

107. Stinson SF, Ryan DP, Hertweck MS, Hardy JD, Hwang-Kow SY, Loosli CG. Epithelial and surfactant changes in influenza pulmonary lesions. Arch Pathol Lab Med 1976; 100: 147-153

108. Stubbig K, Schmidt H, Böhrer H, Huster Th, Bach A, Motsch J. Surfactantapplikation bei akutem Lungenversagen. Anaesthesist 1992; 41: 555-558

109. Suzuki Y, Robertson R, Fujita Y, Grossmann G. Respiratory failure in mice caused by a hybridoma making antibodies to the 15 kDa surfactant apoprotein. Acta Anaesthesiol Scand 1988; 32: 283-289

110. Sweet C, Smith H. Pathogenicity of influenza virus. Microbiol Rev 1980; 44: 303-330

111. Toung TJ, Saharia P, Permutt S, Zuidema GD, Cameron JL. Aspiration pneumonia: beneficial and harmful effects of positive end-expiratory pressure. Surgery 1977; 82: 279-283

112. Weg J, Reines H, Balk R, Tharratt R, Kearney P, Killian T, Scholten D, Zaccardelli D, Horton J, Pattishall E, the Exosurf-ARDS sepsis study group. Safety and efficacy of an aerosolized surfactant (Exosurf) in human sepsis-induced ARDS. Chest 1991; 100 (Suppl): 137S

113. Von Wichert P, Kohl FV. Decreased dipalmitoyllecithin content found in lung specimens from patients with so-called shock-lung. Intensive Care Med 1977; 3: 27-30

114. Wiedemann H, Baugham R, deBoisblanc B, Schuster D, Caldwell E, Weg J, Balk R, Jenkinson S, Wiegelt J, Tharratt R, Horton J, Pattishall E, Long W, the Exosurf ARDS sepsis study group. A

Multicenter trial in human sepsis-induced ARDS of an aerosolized synthetic surfactant (Exosurf). Am Rev Respir Dis 1992; 145: A184

115. Yoneda K, Walzer PD. Mechanism of pulmonary alveolar injury in experimental Pneumocystis carinii pneumonia in the rat. Br J Exp Path 1981; 62: 339-346

116. Zelter M, Escudier J, Hoeffel JM, Murray JF. Effects of aerolized artificial surfactant on repeated oleic acid injury in sheep. Am Rev Respir Dis 1990; 141: 1014-1019

CHAPTER 3

IN VIVO AND IN VITRO INHIBITION OF SURFACTANT FUNCTION BY PROTEIN-RICH PULMONARY EDEMA FLUID

E.P. Eijking, D. Gommers, K.L. So, R. van der Kamp,
M.P.M. de Maat[#] and B. Lachmann

Depts. of Anesthesiology and [#]Internal Medicine II, Erasmus University,
Rotterdam, The Netherlands

Submitted for publication

SUMMARY

Both ARDS and neonatal RDS are characterized by intraalveolar protein-rich edema fluid, capable of inhibiting surfactant function. This study investigated the influence of pulmonary edema fluid raised in rat lungs after hydrochloric acid aspiration and homologous plasma on surfactant function. The first part of the study demonstrated that both edema fluid and plasma, intratracheally instilled in healthy rats, led to respiratory failure as evidenced by decrease of PaO_2 and increase of $PaCO_2$, and to high minimal surface tension of bronchoalveolar lavage material. The second part showed that exogenous surfactant (100 mg/kg), which normally restores gas exchange in surfactant-depleted lungs, failed to do so when mixed with either edema fluid or plasma; these mixtures showed high minimal surface tensions compared to exogenous surfactant alone. In contrast to rats which received plasma, bronchoalveolar lavage fluid of rats which received edema fluid showed a high percentage of neutrophils, suggesting the presence of chemotactic factors in the edema fluid. It is concluded that both edema fluid and plasma have the same inhibitory capacity on surfactant function. However, in these studies the origin of the chemotactic factors present in the edema fluid and the role of the neutrophils have not yet been established.

INTRODUCTION

Both the adult respiratory distress syndrome (ARDS) and the neonatal respiratory distress syndrome (RDS) are characterized by high-permeability pulmonary edema containing plasma-derived proteins. From in vitro studies it is known that these proteins inhibit pulmonary surfactant function in a dose-dependent way (1-8). Also, bronchoalveolar lavage fluid (BAL) and lung homogenates of ARDS and RDS patients demonstrate both quantitative and qualitative changes in phospholipid composition of surfactant and decreased surfactant activity (5,9-12). These findings have led to the rationale for exogenous surfactant therapy in clinical trials.

In neonates exogenous surfactant has been generally accepted for treatment of RDS (for review see [13]). To improve gas exchange in these infants, a single dose of surfactant (approximately 100 mg/kg, depending on the surfactant preparation used) has

proven sufficient (13). However, some infants did not respond to a single dose and required either a higher dose (14), or multiple doses (15) of exogenous surfactant. A few case reports on exogenous surfactant treatment for ARDS have been published; the best results were seen in patients treated with higher surfactant concentrations (approximately 300 mg/kg) (16-18). In both ARDS and RDS lack of response, or only transient response, after a single (low) dose of surfactant could probably be attributed to the alveolar space being filled with protein-rich edema fluid.

In a recent study (19) we reported that in rats suffering from respiratory failure due to hydrochloric acid (HCl) aspiration, exogenous surfactant could not restore gas exchange when given after 90 min. However, when surfactant was given before deterioration of lung function (i.e. within 10 min after HCl aspiration), almost normal gas exchange could be preserved. It was discussed whether failure of exogenous surfactant treatment to restore gas exchange after deterioration of lung function was due to edema fluid forming a mechanical barrier, preventing exogenous surfactant from entering the alveolar space, or surfactant being directly inhibited by plasma proteins. Another possibility could be that edema fluid contains specific mediators capable of inhibiting exogenous surfactant function. For this reason, the present study is designed to investigate the influence of pulmonary edema fluid and homologous plasma on lung function of healthy adult rats after intratracheal instillation. Also, the question is raised whether there is any difference in inhibitory capacity between edema fluid and plasma.

MATERIALS AND METHODS

Exogenous surfactant

The surfactant used in these experiments is a freeze-dried natural surfactant isolated from bovine lungs in basically the same manner as previously described (20). It consists of approximately 90% phospholipids, 1% hydrophobic proteins (SP-B and SP-C), the remainder being other lipids such as cholesterol, glyceride and free fatty acids. There is no SP-A (the largest surfactant-associated protein: molecular weight 26-38 kDa) in this surfactant preparation. In this study surfactant was suspended in saline. The surfactant preparation used has proven to be highly effective in improving gas exchange and lung mechanics in various animal models of respiratory failure of differing etiologies (19,21-

23) and in newborn babies suffering from respiratory failure due to congenital diaphragmatic hernia (24).

Pulmonary edema fluid

This protocol was approved by the Animal Care and Use Committee of the Erasmus University Rotterdam, The Netherlands.

Pulmonary edema was raised in male adult Sprague-Dawley rats (body weight: 300-350 g) after the following procedures (19). In brief: after induction of anesthesia with nitrous oxide, oxygen and halothane (65/33/2%) the animals were tracheotomized and a catheter was inserted into the carotid artery. Anesthesia was maintained with pentobarbital sodium (60 mg/kg, i.p.) and muscle relaxation was attained with pancuronium bromide (0.5 mg/kg, i.m.). The animals were ventilated at the following ventilator settings: pressure-controlled ventilation, FiO_2=1.0, peak airway pressure (P_{peak})=14 cmH_2O, positive end-expiratory pressure (PEEP)=2 cmH_2O, ventilation frequency=30/min and inspiratory/expiratory ratio = 1:2.; a Servo Ventilator 900 C (Siemens-Elema, Solna, Sweden) was used. After reaching steady state (PaO_2>500 mmHg) the animals received HCl intratracheally (0.1 N, 3 ml/kg); P_{peak} was increased to 26 cmH_2O and PEEP to 6 cmH_2O and these ventilator settings were maintained throughout the observation period. Blood samples were taken at regular intervals for measurement of PaO_2 and $PaCO_2$ (ABL 330; Radiometer, Copenhagen, Denmark). After 1-2 hours PaO_2 decreased below 100 mmHg at the used ventilator settings and an amount of 1.5-2.0 ml (4.5-6.0 ml/kg body weight) hemorrhagic edema fluid could be aspirated from the lungs by gentle suction via the trachea cannula. The animals were then sacrificed with an overdose of intra-arterially administered pentobarbital sodium.

Before instillation of HCl, 2 ml of citrated blood was taken from all rats and replaced by saline. Both edema fluid and blood were centrifugated at 2000 g for 15 min to remove all cellular material. The supernatant of all edema samples and plasma were pooled and stored at -20 °C until used for experimental purposes. Before storage, pH of edema fluid and plasma was measured (7.6 and 7.4, respectively). Also, protein concentrations of edema fluid and plasma were measured using a modified Lowry method (25), with bovine serum albumin as standard.

In the edema fluid, clot formation was observed after 30 min at room temperature; this clot was removed from the edema fluid. Because of this observation a quantitative measurement of fibrinogen concentration was performed. Wells of an agarose-plate containing goat antiserum against rat fibrinogen (Nordic Immunological Labs, Tilburg, The Netherlands) were filled with (a) rat plasma at different dilutions, (b) rat serum at different dilutions and (c) rat edema fluid at different dilutions. The proteins were allowed to migrate overnight in an electric field. After precipitation the agarose-plate was stained. The height of the precipitation peak is related to the protein concentration ("rocket immunoelectrophoresis").

Animal studies

Experiment I: To study the effect of intratracheal instillation of edema fluid and homologous plasma on lung function, 24 male Sprague-Dawley rats were used (body weight: 300-350 g). These animals underwent the same anesthesia procedure as described above and the same ventilator settings were used. After reaching steady state (PaO_2> 500 mmHg) the rats were randomly divided into three groups: Group IA (n=8) received edema fluid intratracheally (4 ml/kg); Group IB (n=6) received plasma (4 ml/kg) and Group IC (n=10) received saline (4 ml/kg). P_{peak} was increased to 26 cmH_2O and PEEP to 6 cmH_2O. These ventilator settings were maintained throughout the observation period. Blood samples for measurement of PaO_2 and $PaCO_2$ were taken from the carotid artery of each rat before intratracheal instillation and at 5, 30, 60, 90 120, 150 and 180 min post-instillation. At the end of the experiment (180 min) all rat lungs were lavaged with saline (37 °C; 30 ml/kg) and the animals were then sacrificed as described above.

Experiment II: To study the effect of edema fluid and homologous plasma on an exogenous surfactant preparation 18 male Sprague-Dawley rats were used (body weight 300-350 g). These animals underwent the same anesthesia procedure as described above and the same ventilator settings were used. After reaching steady state (PaO_2>500 mmHg) respiratory failure was induced by bronchoalveolar lavage (BAL) as described by Lachmann and colleagues (26). In brief: lungs were lavaged 6-7 times with warm saline (37 °C; 30 ml/kg) to produce a PaO_2<80 mmHg at P_{peak}=26 cmH_2O and PEEP=6 cmH_2O. In all animals the first lavage was collected and prepared as described below ("Control" lavage). The ventilator settings were unchanged throughout the entire

observation period. Approximately 5 min after PaO_2<80 mmHg animals were randomly divided into three groups: Group IIA (n=6) received surfactant mixed with undiluted edema fluid intratracheally; Group IIB (n=6) received surfactant mixed with undiluted plasma, and Group IIC (n=6) received surfactant mixed with saline. All rats were treated with a surfactant dose of 100 mg/kg; the surfactant preparation used in all groups was suspended in saline at a concentration of 30 mg/ml. Mixtures (v:v=1 ml:1 ml) used in all rats were incubated together for 30 min at 37°C. Blood samples for measurement of PaO_2 and $PaCO_2$ were taken from the carotid artery before BAL and 5 min after the last lavage (directly followed by treatment) and at 5, 30, 60, 90, 120, 150 and 180 min post-treatment. At the end of the experiment rat lungs were lavaged with saline (37°C; 30 ml/kg) and the animals were sacrificed, as described before.

Inhibitory activity of edema fluid

Influence of the edema fluid on the surface tension properties of the exogenous surfactant preparation was investigated using a modified Wilhelmy balance (E. Biegler GmbH, Mauerbach, Austria). In brief: a tight-fitting teflon barrier reduces the surface area of a teflon trough from 100-20% at a cycle speed of 0.33/min. Saline is used as subphase and is kept at 37°C. The force on a platinum slide (1x1 cm), dipped into the subphase, is measured by a force transducer and expressed as surface tension. Further, maximal surface tension is measured at 100% surface area and minimal surface tension at 80% surface-compresion and are expressed as milli Newton/meter (mN/m). Surface tension characteristics of a sample are measured after application on the surface of the saline-filled trough.

In this study the surfactant suspension was used at the same concentration as used in the animal study (30 mg/ml). There were 5 groups of samples for surface activity measurements; each group consisting of 4 samples. In the first group 25 μl of the surfactant suspension is applied to the surface of the saline-filled trough. In the second group 25 μl of the undiluted edema fluid and in the third group 25 μl of undiluted plasma, is applied to the surface. In the fourth group 25 μl of surfactant suspension is mixed with edema fluid (v:v=1:1); in the fifth group 25 μl of surfactant suspension is mixed with plasma (v:v=1:1), and applied to the surface. Maximal and minimal surface tensions are measured after 3 cycles.

Bronchoalveolar lavage fluid

The bronchoalveolar lavage (BAL) samples of all rats (Groups IA-IC, Groups IIA-IIC and the "Control" lavages) were prepared as follows: All BAL samples were centrifugated for 5 min at 400 g. After removal of the erythrocytes by washing with cold H_2O and centrifugation, the cellular sediment was prepared for total cell count and cell differentiation according to standard techniques. The supernatant was centrifugated for 15 min at 2000 g to remove the remaining cell material.

Total protein concentration was measured in all BAL samples using a modified Lowry method (25), with bovine serum albumin as standard.

Surface activity in BAL fluid was measured with the Wilhelmy balance by applying 500 μl of BAL fluid to the surface of the saline-filled trough. Maximal and minimal surface tensions were measured after 3 cycles.

Statistical analysis

All data are expressed as mean ± standard deviation (SD). For PaO_2 and $PaCO_2$ values statistical analysis was performed using repeated measurements ANOVAs, with time as the repeat variable. For analysis of BAL parameters, standard ANOVA procedures were performed. When significant differences between and/or within groups occurred, these differences were further analyzed with a test that compensated for multiple comparison (Student-Newman-Keuls test). Statistical significance was accepted at $p \leq 0.05$.

RESULTS

In vitro studies

The total protein concentration in pooled edema fluid and plasma were 44.5 and 56.6 mg/ml, respectively; pooled edema fluid and rat serum did not contain any fibrinogen.

Both edema fluid and plasma led to inhibition of exogenous surfactant, as demonstrated by an increase of both maximal and minimal surface tensions of the exogenous surfactant preparation (Table 1).

Table 1: Effect of edema fluid and plasma on surface properties of exogenous surfactant.

Sample	n	Surface tension [mN/m]	
		maximal	minimal
Surfactant	4	23.9* ± 3.44	11.6* ± 1.33
Edema	4	61.7 ± 1.45	33.4# ± 1.60
Plasma	4	63.4 ± 2.25	40.0 ± 2.45
Surf + Edema§	4	33.0@ ± 2.05	22.8 ± 1.40
Surf + Plasma§	4	39.9 ± 3.83	21.9 ± 1.66

All data are mean ± SD; *$p<0.05$ between Surfactant and other groups; #$p<0.05$ between Edema and Plasma; @$p<0.05$ between Surf + Edema and Surf + Plasma; §$p<0.05$ between Surf + Edema/Surf + Plasma and Edema/Plasma.

Animal studies

Experiment I: Figure 1 shows PaO_2 values from healthy animals which received either edema fluid, plasma, or saline. Before instillation PaO_2 values were above 500 mmHg in all groups. After receiving edema fluid or plasma PaO_2 values decreased, whereas PaO_2 values of rats receiving saline remained above 500 mmHg. After receiving edema fluid there was a slight increase in $PaCO_2$ values, whereas $PaCO_2$ values did not change in rats receiving plasma; in rats which had received saline there was a slight decrease of $PaCO_2$ values (Fig. 2). For statistically significant differences, see Figures 1 and 2.

Experiment II: The intergroup differences in PaO_2 values both before and after lavage were not statistically different (Fig. 3). After treatment, the PaO_2 values of the rats receiving surfactant mixed with saline increased to pre-lavage values and were significantly higher compared to rats receiving surfactant mixed with edema or plasma.

$PaCO_2$ values of rats receiving surfactant mixed with saline were significantly lower compared to rats receiving surfactant mixed with edema fluid or plasma (Fig. 4).

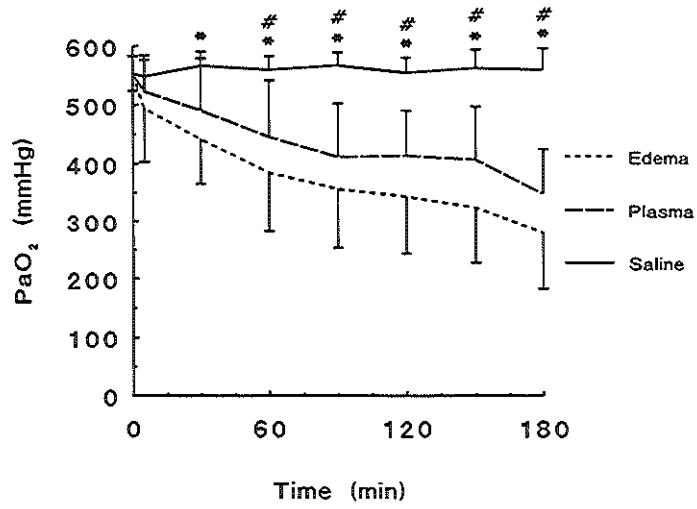


Figure 1. PaO_2 values (mean ± SD) of different groups: healthy rats received edema fluid, plasma or saline intratracheally at 4 ml/kg; *= significant difference between rats which received edema fluid and saline; #= significant difference between rats which received plasma and saline; PaO_2 = arterial oxygen tension.

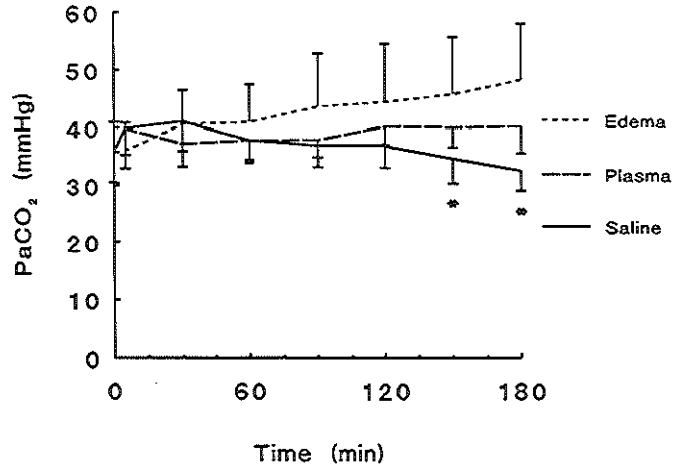


Figure 2. $PaCO_2$ values (mean ± SD) of different groups; for explanation of groups, see Figure 1; *= significant difference between rats which received edema fluid and saline; $PaCO_2$ = arterial carbon dioxide tension.

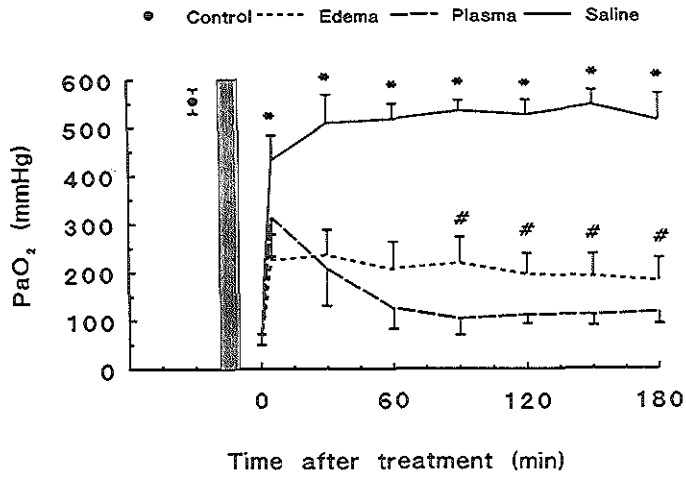


Figure 3. PaO_2 values (mean ± SD) of different groups: after BAL (=grey bar) rats received surfactant (100 mg/kg) mixed with edema fluid, plasma or saline; Control = PaO_2 values before BAL; t=0 indicates PaO_2 values 5 min after BAL, immediately followed by treatment; *= significant difference between rats which received surfactant mixed with saline and other groups; # = significant difference between rats which received surfactant mixed with edema fluid and plasma; PaO_2 = arterial oxygen tension.

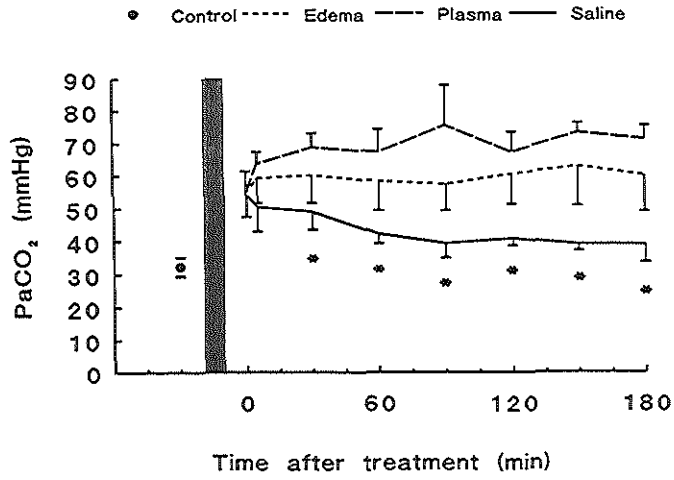


Figure 4. $PaCO_2$ values (mean ± SD) of different groups; for explanation of groups, see Figure 3; *= significant difference between rats which received surfactant mixed with saline and other groups; $PaCO_2$ = arterial carbon dioxide tension.

Bronchoalveolar lavage fluid

Experiment I: BAL fluid of rats receiving edema fluid showed high minimal surface tension compared to lavage fluid from healthy rats (Control) (Table 2). Significantly higher total cell count/ml was seen in BAL fluid from rats which received edema fluid, compared to all other rats; also, significantly low macrophage percentage and high granulocyte percentage was seen in rats which received edema fluid, compared to the other rats (Table 3).

Table 2: BAL parameters from Experiment I.

Group	n	Recovery [%]	Protein [mg/ml]	Surface tension [mN/m]	
				maximal	minimal
Edema	8	96.7 ± 2.36	6.79* ± 0.78	59.1 ± 3.00	28.9@ ± 3.36
Plasma	6	95.0 ± 4.47	6.01# ± 0.55	57.3 ± 3.34	26.2 ± 2.93
Saline	10	96.3 ± 4.33	0.48 ± 0.51	61.8 ± 3.40	25.8 ± 3.12
Control	8	95.6 ± 6.43	0.23 ± 0.07	61.2 ± 6.72	23.3 ± 3.63

All data are mean ± SD; Control = BAL fluid of healthy control rats; *$p<0.05$ between rats receiving edema fluid and saline/Control; #$p<0.05$ between rats receiving plasma and saline/Control; @$p<0.05$ between rats receiving edema fluid and Control.

Table 3: Cellular parameters in BAL from Experiment I.

Group	Cell count [x10⁴/ml]	Macrophages# [%]	Granulocytes@ [%]	Lymphocytes [%]	Viability [%]
Edema	17.2* ± 4.44	11.7 ± 4.50	87.7 ± 4.50	0.70 ± 0.47	98.6 ± 0.83
Plasma	5.64 ± 2.77	42.0 ± 9.86	56.5 ± 10.5	1.53 ± 1.54	98.9 ± 0.79
Saline	2.68 ± 1.57	61.0 ± 7.18	36.5 ± 5.94	2.50 ± 2.06	95.0 ± 1.73
Control	2.54 ± 1.46	96.2 ± 3.16	2.51 ± 2.09	1.31 ± 1.15	94.6 ± 3.75

All data are mean ± SD; *$p<0.05$ between rats receiving edema fluid and other groups; #,@$p<0.05$ between all groups; viability = percentage of living leucocytes.

Table 4: BAL parameters from Experiment II.

Group	n	Recovery [%]	Protein [mg/ml]	Surface tension [mN/m]	
				maximal	minimal
Surfactant + edema	5	100 ± 0	5.35 ± 0.33	60.1 ± 1.98	21.4 ± 2.08
Surfactant + plasma	5	100 ± 0	5.09 ± 0.74	57.1 ± 1.44	21.1 ± 1.24
Surfactant + saline	6	100 ± 0	1.86* ± 1.11	55.1 ± 3.97	19.6 ± 2.27

All data are mean ± SD; *$p<0.05$ between rats which receiving surfactant + saline and other groups.

Table 5: Cellular parameters in BAL from Experiment II.

Group	Cell count [x10⁴/ml]	Macrophages [%]	Granulocytes [%]	Lymphocytes [%]	Viability [%]
Surfactant + edema	12.4* ± 4.89	20.5# ± 9.50	79.0# ± 9.67	0.50 ± 0.50	99.9 ± 0.13
Surfactant + plasma	3.88 ± 1.44	43.3 ± 5.44	52.3 ± 5.44	1.00 ± 0.82	85.1 ± 8.80
Surfactant + saline	4.00 ± 1.31	65.0 ± 21.6	34.0 ± 2.02	1.10 ± 1.44	89.7 ± 6.95

All data are mean ± SD; *$p<0.05$ between rats receiving surfactant + edema and other groups; #$p<0.05$ between rats receiving surfactant + edema and surfactant + saline; viability = percentage of living leucocytes.

Experiment II: There was no difference in surface tension properties between all groups (Table 4). Total cell count/ml was significantly higher in BAL fluid from rats which received surfactant mixed with edema fluid, compared to all other groups; BAL fluid from rats which received surfactant mixed with edema fluid demonstrated a significantly low macrophage percentage and significantly high granulocyte percentage, compared to BAL fluid from rats which received surfactant mixed with saline (Table 5).

DISCUSSION

The first part of this study (Experiment I) demonstrated that intratracheal instillation of both protein-rich edema fluid and homologous plasma in healthy rats were capable of disturbing gas exchange across the alveolar-capillary membrane at the used ventilator settings. BAL fluid of rats receiving edema fluid showed high minimal surface tension compared to lavage fluid from healthy controls. These results suggest interference of both edema fluid and plasma with the surfactant layer of the lungs.

The BAL fluid of healthy rats receiving edema fluid contained a significantly

higher percentage of (neutrophilic) granulocytes compared to BAL fluid of other groups. These findings suggest attraction of neutrophils into the alveoli, probably due to chemotactic factors present in the edema fluid, as is also described in animal lungs after HCl aspiration (27,28). These neutrophils are capable of producing toxic oxygen radicals and specific proteases (29,30), leading to further destruction of the alveolar-capillary membrane and, perhaps, also to direct damage of the surfactant system (31). This not only suggests that certain factors in edema fluid in ARDS are capable of interfering with the surfactant layer, it also appears that high-permeability edema fluid itself can induce and maintain an ARDS-like situation in healthy animals. This observation could explain why it is difficult to treat the respiratory insufficiency when the cause of the ARDS has been treated successfully: the ARDS may support itself.

The second part of this study (Experiment II) was designed to investigate whether edema fluid and homologous plasma are capable of inhibiting the ability of a therapeutical dosage of an exogenous surfactant preparation to restore gas exchange in animals suffering from acute respiratory failure. For this purpose the lung lavage model was used. This model has proven useful for a variety of experimental purposes, including e.g. testing of different surfactant preparations and demonstrating that exogenous surfactant restores blood gases to normal (26,32,33). The dose of surfactant (100 mg/kg) used in this study has proven to be sufficiently high to restore lung function in rats suffering from respiratory failure after bilateral whole lung lavage (33). However, surfactant at this concentration mixed with edema fluid or plasma was not able to restore lung function. This observation could not be explained by a fluid effect, per sé: surfactant mixed with saline was capable to restore gas exchange to normal values. Surface tension measurements of these mixtures showed that both maximal and minimal surface tensions of surfactant mixed with edema fluid or plasma were significantly higher compared to surfactant alone. BAL fluid of rats receiving surfactant mixed with edema fluid again had a significantly higher percentage of neutrophils compared to BAL fluid of other groups.

It is known that surfactant function can be inhibited in a dose-dependent way by whole blood, plasma, serum and different plasma proteins such as fibrinogen, fibrin monomers, albumin, globulin, hemoglobin and cell membrane lipids (1-4,7,8). Also, it has been demonstrated that phospholipases can inhibit surfactant function (34). A specific plasma-derived surfactant inhibitor (MW =110.000 Da, unreduced) has been isolated

from the alveolar washes of prematurely delivered and ventilated lambs, and in alveolar washes of infants suffering from RDS (5,6).

The mechanism by which plasma-derived proteins inhibit surfactant function is not clear. Balis and colleagues have postulated two different types of surfactant inhibition (1). They demonstrated that inhibition of surfactant by serum could be reversed by centrifugating the surfactant-serum mixture: the sediment revealed normal surfactant function. Mixing surfactant with plasma allowed clot formation to occur; this "coagulative type" of inhibition, could not be reversed by centrifugation. The existence of this type of inhibition seems plausible, because of the fact that alveoli of infants with RDS are covered with hyaline membranes, containing fibrin and cellular debris (35). Also, as already mentioned, fibrinogen and fibrin monomers are potent surfactant inhibitors (2,7,8). As mentioned in the section "Materials and Methods" clot formation was observed in the edema fluid. One explanation could be that the edema fluid resembles (non-citrated) plasma, resulting in clot formation. Another explanation could be that the (endogenous) surfactant reveals thromboplastic activity (8). In the present study it appears that fibrinogen is not essential for inhibition of surfactant function, since the edema fluid did not contain any fibrinogen. However, no measurements on fibrin-breakdown products were performed.

Other investigators have hypothesized that inhibition of surfactant by plasma derived proteins is due to competition for space at the air-liquid interphase. Holm and colleagues reported that, at high surfactant concentrations the inhibitory effect of high concentrations of plasma protein or membrane lipids was abolished (3,4). Other studies confirm the probable existence of a competition mechanism: after centrifugation of surfactant-inhibitor mixtures derived from BAL fluid of animals treated with surfactant, or after centrifugation of surfactant-protein mixtures, the sediments containing surfactant revealed normal surface tension characteristics compared with pre-centrifugation (1,4,5). Also, when these BAL fluids or the surfactant-protein mixtures were investigated for surface tension properties, it could be demonstrated that after a certain number of cycles the minimal surface tension decreased from initial high levels to low levels (2,6,7).

If the inhibition of surfactant function is dose-dependent, this would be of great importance for treatment of ARDS and neonatal RDS. Namely, in this case it would seem to be merely a case of attaining a high surfactant/inhibitor (S/I) ratio. Thus,

whether the lungs are filled with plasma proteins or not, intratracheal instillation of sufficiently high dosage of surfactant should restore lung function. In a recent study, surfactant at 200 mg/kg was not able to restore gas exchange in rats suffering from respiratory failure due to HCl aspiration (19). In another study, it was demonstrated that surfactant instillation at very high doses (280-350 mg/kg) was able to restore gas exchange in guinea pigs suffering from severe respiratory failure due to high permeability pulmonary edema after intravenous instillation of anti-lung serum (36). Recently, Kobayashi and colleagues investigated the capability of surfactant mixed with edema fluid at several ratios to restore lung function in immature rabbit fetuses, as measured by tidal volume at preset insufflation pressures (37). It was demonstrated that surfactant (25 mg/ml) mixed with edema fluid at a protein to lipid ratio (P/L ratio) of 2.2 was capable of restoring lung function, whereas surfactant mixed with edema fluid at P/L ratio of 11.2, was not. Surface tension properties of these mixtures demonstrated high minimal surface tensions at $P/L \geq 3.4$ and low surface tensions at $P/L \leq 1.8$. Another way to accomplish a high S/I ratio would be to first lavage the lungs, followed by surfactant treatment (38,39). In a recent study we demonstrated that lung function could be restored in rats suffering from severe respiratory failure due to HCl aspiration after lavaging the lungs with a diluted surfactant suspension (39). From these results one can speculate that in the present study a larger amount of exogenous surfactant would perhaps be able to overcome the inhibition of both edema fluid and plasma. Also, in the case of edema fluid, perhaps anti-inflammatory drug therapy could be used to prevent attraction of neutrophils into the lungs.

In conclusion: it has been demonstrated that both edema fluid and plasma interfere with the surfactant layer, probably by direct inhibition due to plasma-derived proteins. However, in the case of edema fluid some inflammatory agents could, in part, be responsible for the inhibition of surfactant function. The edema fluid and plasma were also capable to inhibit a therapeutical dosage of an exogenous surfactant preparation to restore gas exchange in surfactant depleted animals. Since inhibition of surfactant is dose-dependent it is proposed to attain a high S/I ratio by means of increasing the amount of surfactant, or decreasing the inhibitory effect via BAL (with saline or a diluted surfactant suspension) and giving surfactant at sufficiently high concentration.

REFERENCES

1. Balis JU, Shelley SA, McCue MJ, Rappaport ES. Mechanisms of damage to the lung surfactant system. Ultrastructure and quantitation of normal and in vitro inactivated lung surfactant. Exp Mol Pathol 1971; 14: 243-62.
2. Fuchimukai T, Fujiwara T, Takahashi A, Enhorning E. Artificial pulmonary surfactant inhibited by proteins. J Appl Physiol 1987; 62: 429-37.
3. Holm BA, Notter RH. Effects of hemoglobin and cell membrane lipids on pulmonary surfactant activity. J Appl Physiol 1987; 63: 1434-42.
4. Holm BA, Enhorning G, Notter RH. A biophysical mechanism by which plasma proteins inhibit lung surfactant activity. Chem Phys Lipids 1988; 49: 49-55.
5. Ikegami M, Jacobs H, Jobe A. Surfactant function in respiratory distress syndrome. J Pediatr 1983; 102: 443-7.
6. Ikegami M, Jobe A, Jacobs H, Lam R. A protein from airways of premature lambs that inhibits surfactant function. J Appl Physiol 1984; 57: 1134-42.
7. Seeger W, Stöhr G, Wolf HRD, Neuhof H. Alteration of surfactant function due to protein leakage: special interaction with fibrin monomer. J Appl Physiol 1985; 58: 326-38.
8. Taylor Jr. FB, Abrams ME. Effect of surface active lipoprotein on clotting and fibrinolysis, and of fibrinogen on surface tension of surface active lipoprotein (with a hypothesis on the pathogenesis of pulmonary atelectasis and hyaline membrane in respiratory distress syndrome of the newborn). Am J Med 1966; 40: 346-50.
9. Avery ME, Mead J. Surface properties in relation to atelectasis and hyaline membrane disease. Am J Dis Child 1959; 97: 517-23.
10. Hallman M, Spragg R, Harrell JH, et al. Evidence of lung surfactant abnormality in respiratory failure. Study of bronchoalveolar lavage phospholipids, surface activity, phospholipase activity, and plasma myoinositol. J Clin Invest 1982; 70: 673-83.
11. Gregory TJ, Longmore WJ, Moxley MA, et al. Surfactant chemical composition and biophysical activity in acute respiratory distress syndrome. J Clin Invest 1991; 88: 1976-81.
12. Lachmann B, Danzmann E. Adult respiratory distress syndrome. In: Robertson B, Van Golde LMG, Batenburg JJ, eds. Pulmonary surfactant. Amsterdam: Elsevier Science Publishers, 1984: 505-48.
13. Merritt TA, Hallman M, Spragg R, et al. Exogenous surfactant treatment for neonatal respiratory distress syndrome and their potential role in the adult respiratory distress syndrome. Drugs 1989; 38: 591-611.
14. Konishi M, Fujiwara T, Naito T, et al. Surfactant replacement therapy in neonatal respiratory distress syndrome: A multi-centre, randomized clinical trial: comparison of high- versus low-dose of Surfactant TA. Eur J Pediatr 1988; 147: 20-5.
15. Dunn MS, Shennan AT, Possmayer F. Single- versus multiple-dose surfactant replacement therapy

in neonates of 30 to 36 weeks' gestation with respiratory distress syndrome. Pediatrics 1990; 86: 564-71.

16. Lachmann B. The role of pulmonary surfactant in the pathogenesis and therapy of ARDS. In: Vincent JL, ed. Update in intensive care and emergency medicine. Berlin: Springer-Verlag, 1987: 123-34.
17. Nosaka S, Sakai T, Yonekura M, Yoshikawa K. Surfactant for adults with respiratory failure. Lancet 1990; 1: 947-8.
18. Richman PS, Spragg RG, Robertson B, et al. The adult respiratory distress syndrome: first trials with surfactant replacement. Eur Respir J 1989; 2 (Suppl 3): 109s-11s.
19. Eijking EP, Gommers D, So KL, et al. Prevention of respiratory failure after hydrochloric acid aspiration by intratracheal surfactant instillation in rats. Anesth Analg 1993; 76: 472-7.
20. Metcalfe IL, Enhorning G, Possmayer F. Pulmonary surfactant-associated proteins: their role in the expression of surface activity. J Appl Physiol 1980; 49: 34-41.
21. Van Daal GJ, So KL, Gommers D, et al. Intratracheal surfactant administration restores gas exchange in experimental adult respiratory distress syndrome associated with viral pneumonia. Anesth Analg 1991; 72: 589-95.
22. Van Daal GJ, Bos JAH, Eijking EP, et al. Surfactant replacement therapy improves pulmonary mechanics in end-stage influenza A pneumonia in mice. Am Rev Respir Dis 1992; 145: 859-63.
23. Eijking EP, Van Daal GJ, Tenbrinck R, et al. Effect of surfactant replacement on pneumocystis carinii pneumonia in rats. Intensive Care Med 1991; 17: 475-8.
24. Bos AP, Tibboel D, Hazebrock FWJ, et al. Surfactant replacement therapy in high-risk congenital diaphragmatic hernia. Lancet 1991; (1) 338: 1279.
25. Markwell MAK, Haas SM, Bieber LL, Tolbert NE. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem 1978; 87: 206-10.
26. Lachmann B, Robertson B, Vogel J. In vivo lung lavage as an experimental model of the respiratory distress syndrome. Acta Anaesthesiol Scand 1980; 24: 231-6.
27. Greenfield LJ, Singleton RP, McCaffree DR, Coalson JJ. Pulmonary effects of experimental graded aspiration of hydrochloric acid. Ann Surg 1969; 170: 74-86.
28. Parsons PE, Fowler AA, Hyers TM, Henson PM. Chemotactic activity in bronchoalveolar lavage fluid from patients with adult respiratory distress syndrome. Am Rev Respir Dis 1985; 132: 490-3.
29. Cochrane CG, Spragg R, Revak SD. Pathogenesis of the adult respiratory distress syndrome: evidence of oxidant activity in bronchoalveolar lavage fluid. J Clin Invest 1983; 71: 754-61.
30. Weiss SJ. Tissue destruction by neutrophils. N Engl J Med 1989; 320: 365-76.
31. Ryan SF, Ghassibi Y, Liau DF. Effects of activated polymorphonuclear leukocytes upon pulmonary surfactant in vitro. Am J Respir Cell Mol Biol 1991; 4: 33-41.
32. Berggren P, Lachmann B, Curstedt T, et al. Gas exchange and lung morphology after surfactant

replacement in experimental adult respiratory distress syndrome induced by repeated lung lavage. Acta Anaesthesiol Scand 1986; 30: 321-8.

33. Robertson B, Lachmann B. Experimental evaluation of surfactants for replacement therapy. Exp Lung Res 1988; 14: 279-310.

34. Holm BA, Keicher L, Liu M, et al. Inhibition of pulmonary surfactant function by phospholipases. J Appl Physiol 1991; 71: 317-21.

35. Gitlin D, Craig JM. The nature of the hyaline membrane in asphyxia of the newborn. Pediatrics 1956; 17: 64-71.

36. Lachmann B, Hallman M, Bergmann KC. Respiratory failure following anti-lung serum: study on mechanisms associated with surfactant system damage. Exp Lung Res 1987; 12: 163-80.

37. Kobayashi T, Nitta K, Ganzuka M, et al. Inactivation of exogenous surfactant by pulmonary edema fluid. Pediatr Res 1991; 29: 353-6.

38. Kobayashi T, Ganzuka M, Tanigushi J, et al. Lung lavage and surfactant replacement for hydrochloric acid aspiration in rabbits. Acta Anaesthesiol Scand 1990; 34: 216-21.

39. So KL, Eijking EP, Gommers D, et al. Different surfactant treatment strategies for respiratory failure induced by hydrochloric acid aspiration in rats. Intensive Care Med 1992; 18 (Suppl 2): S111.

CHAPTER 4

IN VIVO EVALUATION OF THE INHIBITORY CAPACITY OF HUMAN PLASMA ON EXOGENEOUS SURFACTANT FUNCTION

B. Lachmann, E.P. Eijking, K.L. So and D. Gommers

Dept. of Anesthesiology, Erasmus University,
Rotterdam, The Netherlands

Submitted for publication

SUMMARY

Objective: The adult respiratory distress syndrome (ARDS) and neonatal respiratory distress syndrome (RDS) are characterized by high permeability pulmonary edema which contains plasma-derived proteins inhibiting pulmonary surfactant function. Currently, discussion continues as to what dose of surfactant is required for treatment of these syndromes.

Design: The purpose of this study was to investigate the amount of exogenous surfactant needed to overcome the inhibitory components in human plasma. Male adult rats suffering from respiratory failure due to surfactant depletion after whole-lung lavage received human plasma (4 ml/kg body weight) mixed with surfactant at different concentrations, intratracheally. Rats receiving surfactant only at different concentrations served as controls. Blood gas analysis was performed.

Measurements and results: It was demonstrated that plasma (4 ml/kg ≈ 280 mg plasma proteins/kg) mixed with surfactant at 300 mg/kg was able to increase and maintain PaO_2 at normal values. Plasma mixed with surfactant at 100 mg/kg, after initial restoration of blood gases, showed deterioration of PaO_2 values. Plasma mixed with surfactant at a dose of 50 mg/kg did not improve PaO_2, whereas surfactant at 50 mg/kg, without plasma, restored blood gases to pre-lavage values.

Conclusion: It is concluded that approximately 1 mg surfactant phospholipids is required to overcome the inhibitory effect of approximately 1 mg plasma proteins. For clinical practice this means that an excess of surfactant should be given, or repeatedly be substituted ("titrated") at low concentrations, until blood gases improve.

INTRODUCTION

The adult respiratory distress syndrome (ARDS) and neonatal respiratory distress syndrome (RDS) are both characterized by respiratory failure and require therapy consisting of intubation and mechanical ventilation with high oxygen concentrations. RDS is characterized by primary surfactant deficiency due to immaturity of the lungs, while in ARDS high permeability pulmonary edema exists, containing plasma-derived proteins which inhibit pulmonary surfactant function [1-7]. Due to impaired surfactant function,

surface tension at the air-liquid interphase on the alveolar walls is increased, leading to increased suction force across the alveolar-capillary membrane [8]. This results in further intra-alveolar accumulation of protein-rich edema fluid.

Several clinical trials have reported successful treatment with intratracheal surfactant instillation in prematures suffering from RDS [9-14]. In these studies, however, some infants did not respond to a single dose of surfactant and some infants had an only transient improvement of lung function [10,11]. One reason for this could be that surfactant function is inhibited by plasma-derived proteins present in intra-alveolar edema fluid.

A few reports on ARDS patients treated with surfactant have been published. Although the results from these studies are not consistent, the best results were seen in those patients treated with higher surfactant concentrations [15-19].

Currently, discussion continues as to what dose of surfactant should be used in prematures with established RDS and what dose should be used for treatment of ARDS. To investigate this, a study was performed in an animal model of respiratory failure induced by bronchoalveolar lavage (BAL). This model has proven useful for a variety of experimental purposes, including e.g. testing of different surfactant preparations and demonstrating that exogenous surfactant restores blood gases to normal [20-22]. After respiratory failure was established, animals received plasma mixed with surfactant at different concentrations. Plasma was used to simulate protein-rich edema, an established characteristic of ARDS. Instead of lung mechanics, blood gas measurements were measured, since these have proven to be more sensitive to therapeutic interventions in this model [23].

MATERIALS AND METHODS

Exogenous surfactant

The surfactant used in these experiments was a freeze-dried natural surfactant isolated from bovine lungs in basically the same manner as previously described [24]. It consists of approximately 90% phospholipids, 1% hydrophobic proteins (SP-B and SP-C), the remainder being other lipids such as cholesterol, glyceride and free fatty acids. There is no SP-A in this surfactant preparation. This surfactant preparation has proven to be

highly effective in improving gas exchange and lung mechanics in various animal models of respiratory failure of differing etiologies [25-27] and in newborn babies suffering from respiratory failure due to congenital diaphragmatic hernia [28].

Animal study

The protocol was approved by the Animal Care and Use Committee of the Erasmus University Rotterdam.

The studies were performed in 45 male adult Sprague-Dawley rats (body weight 300-350 g). After induction of anesthesia with nitrous oxide, oxygen and halothane (65/33/2%) the animals were tracheotomized and a catheter was inserted into the carotid artery. Anesthesia was maintained with pentobarbital sodium (60 mg/kg, i.p.) and muscle relaxation was attained with pancuronium bromide (0.5 mg/kg, i.m.). The rats were ventilated with a Servo Ventilator 900C (Siemens-Elema, Solna, Sweden) at the following ventilator settings: pressure-controlled ventilation, $F_iO_2 = 1.0$, ventilation frequency=30/min, peak airway pressure (P_{peak}) = 14 cmH_2O, positive end-expiratory pressure (PEEP) = 2 cmH_2O and inspiratory/expiratory ratio=1:2.

After reaching steady state ($PaO_2 > 500$ mmHg) respiratory failure was induced by BAL as described by Lachmann et al [20]. In brief: lungs were lavaged 6-7 times with warm saline (37°C; 30 ml/kg) to produce a $PaO_2 < 80$ mmHg at $P_{peak} = 26$ cmH_2O and PEEP=6 cmH_2O. These ventilator settings were unchanged throughout the entire observation period.

Approximately 5 min after $PaO_2 < 80$ mmHg animals were randomly divided into seven groups: Groups I, II, and III (n=6, n=7 and n=7, respectively) received undiluted citrated pooled human plasma (4 ml/kg) mixed with surfactant at concentrations of 50, 100 and 300 mg phospholipids/kg body weight, respectively; Groups IV, V and VI (n=6, n=6 and n=7, respectively) received surfactant suspended in saline at concentrations of 25, 50 and 100 mg phospholipids/kg body weight, respectively (total amount of surfactant suspension was 4 ml/kg); Group VII (n=6) received undiluted citrated pooled human plasma (4 ml/kg).

Blood samples for measurement of PaO_2 and $PaCO_2$ were taken from the carotid artery before BAL and 5 min after the last lavage (directly followed by treatment) and at 5, 30, 60, 90, 120, 150 and 180 min post-treatment (ABL 330; Radiometer, Denmark).

The animals were then sacrificed with an overdose of intra-arterially administered pentobarbital sodium.

Statistical analysis

All data are expressed as mean ± standard deviation (SD). Statistical analysis of data was performed using repeated measurements Anovas, with time as the repeat variable. When differences between and/or within groups occurred, these differences were further analyzed with a test that compensated for multiple comparison (Student-Newman-Keuls test). Statistical significance was accepted at $p \leq 0.05$.

RESULTS

Figures 1A and 1B show PaO_2 values for all groups. The intergroup differences both before and after lavage are not statistically different. After treatment, the PaO_2 values of groups receiving surfactant only at 50 and 100 mg/kg (Groups V and VI, respectively) increase to pre-lavage values and remain high during the whole observation period and the differences between these groups are not significant; for this reason PaO_2 values of these two groups are used for statistical comparison with other groups. Surfactant only at 25 mg/kg (Group IV), after initial improvement to pre-lavage values, did not stabilize PaO_2 values over the whole observation period; the difference with Groups V/VI was significant after 60 min. PaO_2 values of rats receiving plasma (4 ml/kg) mixed with surfactant at 100 and 300 mg/kg (Groups II and III, respectively) also increase to normal values. Immediately after treatment there is no significant difference in PaO_2 values between Groups II and III and Groups V/VI. However, PaO_2 values of Group II decrease significantly 60 min after treatment compared to Group III. PaO_2 values of Group III remain at high levels throughout the observation period and are not significantly different from Groups V/VI. PaO_2 values of rats receiving plasma mixed with surfactant at 50 mg/kg (Group I) did not increase significantly. Rats receiving plasma only (Group VII) died within 60 min.

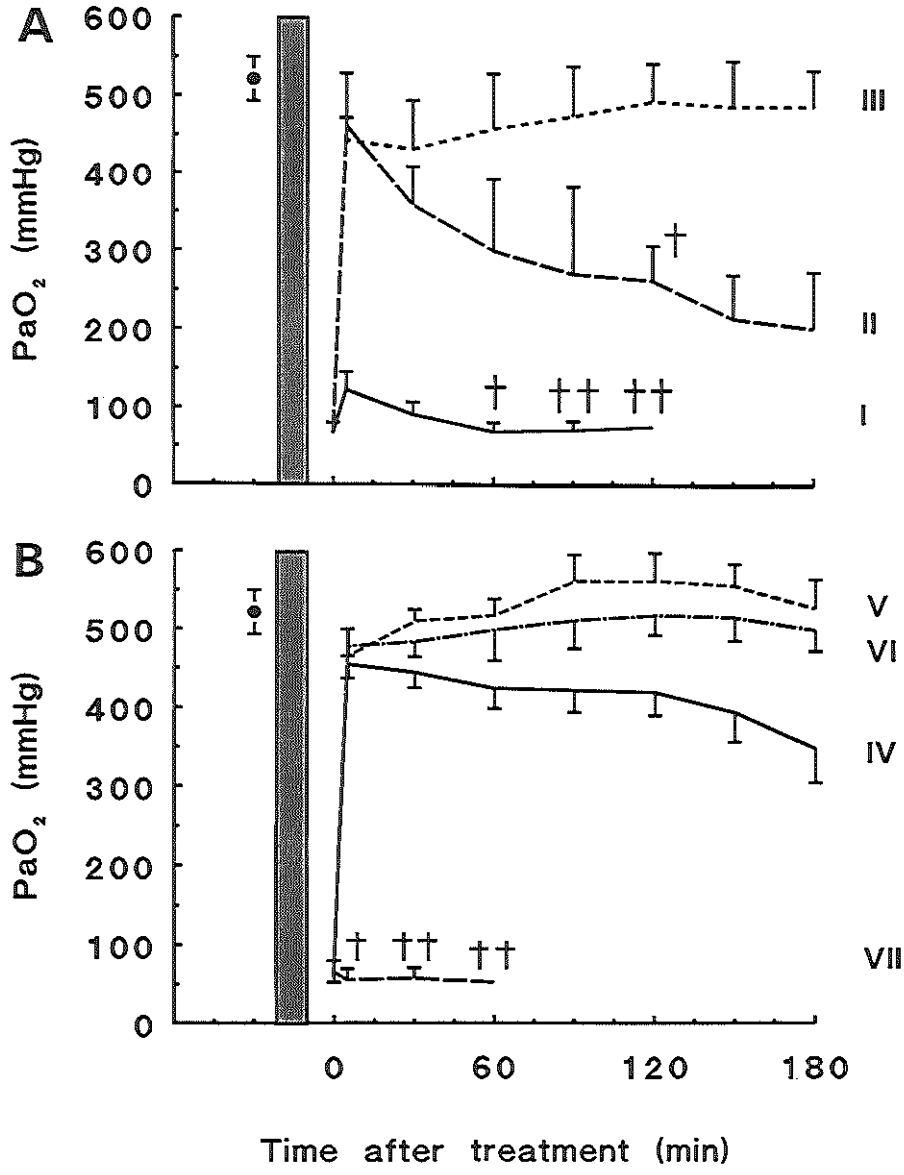


Figure 1. PaO_2 values (mmHg; mean ± SD) before (•) and after BAL (=grey bar) in different treatment groups: Groups I, II, and III received plasma (4 ml/kg) mixed with surfactant at 50, 100 and 300 mg/kg, respectively (A); Groups IV, V and VI received surfactant only at 25, 50 and 100 mg/kg, respectively and Group VII received plasma only (4 ml/kg) (B); t=0 indicates the PaO_2 values 5 min after BAL, immediately followed by treatment; † = one rat died; PaO_2 = arterial oxygen tension.

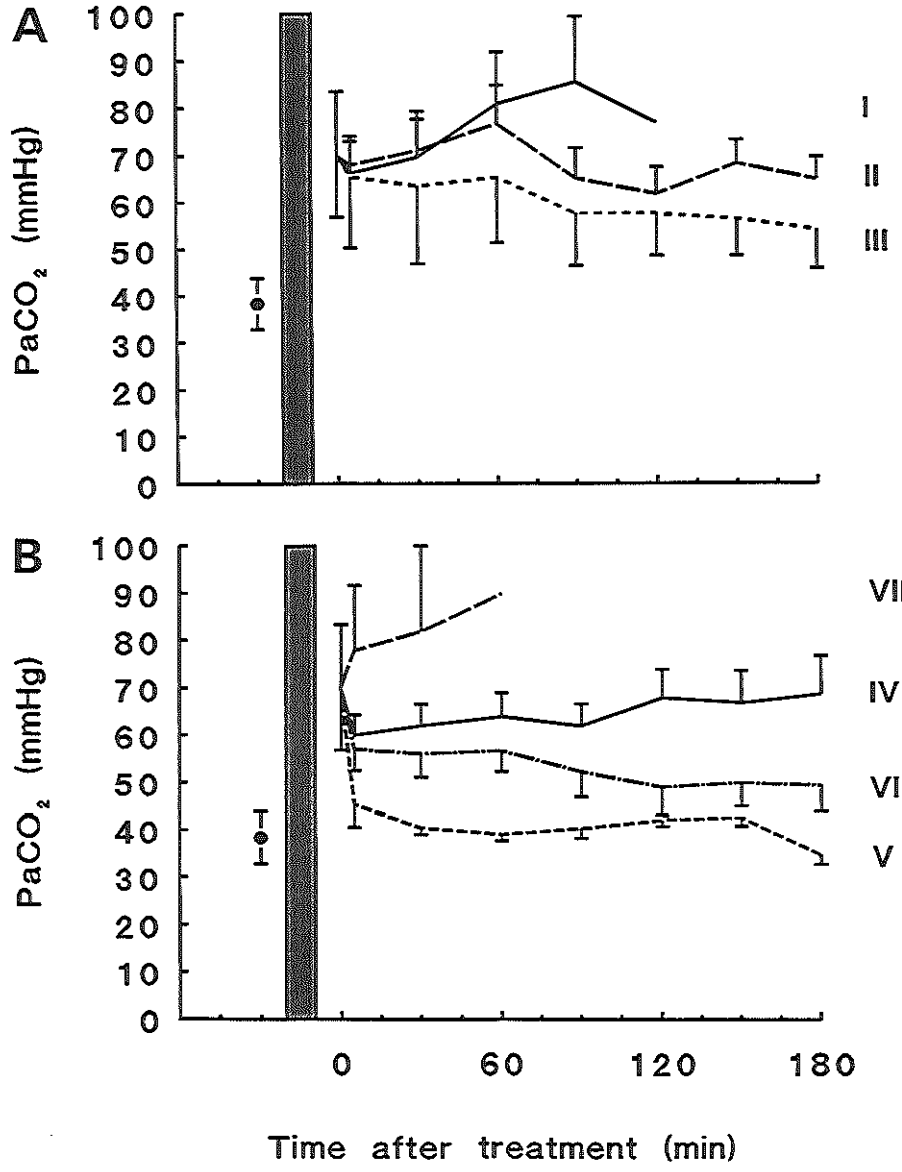


Figure 2. $PaCO_2$ values (mmHg; mean ± SD) before (•) and after BAL (=grey bar) in different treatment groups: for explanation of groups, see Figure 1; $PaCO_2$ = arterial carbon dioxide tension.

Figures 2A and 2B show the $PaCO_2$ values for all groups. The intergroup differences both before and after lavage are not statistically different. $PaCO_2$ values of rats receiving surfactant only at 50 or 100 mg/kg do not differ significantly, and are used for comparison with other groups. $PaCO_2$ values of Groups I, II and VII differ significantly from Groups V/VI. There is no significant difference in $PaCO_2$ values between Group III and Groups V/VI. There are no significant differences in $PaCO_2$ values between Groups II and III.

DISCUSSION

It has been established that in both ARDS and RDS the alveolar-capillary membrane is highly permeable to plasma proteins, leaking from the circulation into the alveolar space, resulting in edema formation [2,29,30]. BAL material of patients suffering from these syndromes demonstrates quantitative and qualitative changes in phospholipid composition of surfactant, and contains high protein concentrations [2,30-33]. Saline extracts of lung minces from infants who died from RDS and from adults with ARDS contained less phospholipids and had higher minimal surface tension than similar extracts from lungs of patients who died from other causes [15,34]. Several clinical studies have been performed treating infants suffering from RDS with intratracheal surfactant instillation [9-14]. The surfactant preparations used varied from natural surfactant (e.g. CLSE, Curosurf, Surfactant TA and human surfactant) to synthetic surfactant preparations (ALEC, Exosurf). The doses used in these clinical trials varied from low (60 mg/kg) to high (200 mg/kg). It has been reported that some infants did not respond to, or had an only transient improvement after a single treatment of low-dose surfactant [10,11]. It has been suggested that in these patients surfactant was gradually inhibited by proteins present in the pulmonary edema fluid. This hypothesis could be confirmed in other clinical studies in which better clinical outcome was seen in infants treated with either a higher dose of surfactant [11] or by treatment with multiple doses [14]. Only a few patients with ARDS have been treated with surfactant. Although the results from these studies are not consistent, the best results were seen in patients treated with higher surfactant concentrations [15-19].

After treatment of RDS and ARDS with a low dose of surfactant the reason for

lack of response, or only transient improvement, is attributed to the alveolar space in these patients being filled with protein-rich edema fluid. Clinical and animal studies have established that these plasma-derived proteins inhibit surfactant [1-7,29,30,35-39]. Surface tension studies have shown that several plasma proteins inhibit surfactant in a dose-dependent manner [7,36,37,40-45]. It is also established that some surfactant preparations, especially synthetic surfactants, are more sensitive to the inhibitory effect of plasma-derived proteins than other preparations [46-48]. However, in recent studies it was demonstrated that addition of surfactant-associated proteins increases resistance of synthetic surfactants to inhibition of plasma proteins [49,50]. In physiological studies using excised lungs it was shown that intratracheal instillation of hemoglobin, albumin or membrane lipids decreased lung compliance, which could be reversed by intratracheal surfactant instillation [42].

The mechanism by which plasma derived proteins inhibit surfactant function is not clear. Balis and colleagues [51] have postulated two different types of surfactant inhibition. They demonstrated that inhibition of surfactant by serum could be reversed by centrifugating the surfactant-serum mixture: the sediment revealed normal surfactant function. Mixing surfactant with plasma allowed clot formation to occur; this "coagulative type" of inhibition could not be reversed by centrifugation. Seeger and colleagues reported that fibrin monomers are potent inhibitors of surfactant; inhibition of surfactant could partially be reversed by adding plasmin to the fibrin-surfactant mixture [7]. Other investigators have hypothesized that inhibition of surfactant by plasma derived proteins is due to competition for space at the air-liquid interphase. Holm and colleagues reported that at high surfactant concentrations the inhibitory effect of high concentrations of plasma protein or membrane lipids is abolished [42,43]. Other studies confirm the probable existence of a competition mechanism: after centrifugation of surfactant-inhibitor mixtures derived from BAL fluid of animals treated with surfactant or after centrifugation of surfactant-protein mixtures, the sediments containing surfactant revealed normal surface tension characteristics compared with before centrifugation [2,35,38,43,51]. Also, when these BAL fluids or the surfactant-protein mixtures were investigated for surface tension properties, it could be demonstrated that after a certain number of cycles the minimal surface tension decreased from initially high levels to low levels [7,36,39,40]. These findings probably indicate that, if there is any chemical

interaction between proteins and surfactant, this interaction is not strong.

If the mechanism of competition between surfactant and plasma proteins is correct, then this would be of great importance for treatment of ARDS and neonatal RDS. Namely, whether the lungs are filled with plasma-derived proteins or not, intratracheal instillation of sufficiently high doses of surfactant should restore lung function. Surfactant treatment of animals with enormous amounts of pulmonary edema caused by prolonged exposure of 100% oxygen or hydrochloric acid (HCl) aspiration does not seem to have any positive influence on restoring gas exchange at the concentrations used [1,4]. Kobayashi and colleagues have demonstrated that surfactant treatment was only able to restore gas exchange in rabbits suffering from respiratory failure after HCl aspiration after lungs had been lavaged, this way removing proteins from the alveolar space [4]. This strongly suggests that a more favorable surfactant/inhibitor ratio was established after lung lavage followed by surfactant treatment. In another study, surfactant instillation at very high doses (280-350 mg/kg) was able to restore gas exchange in guinea pigs suffering from severe respiratory failure due to high permeability pulmonary edema after intravenous instillation of anti-lung serum [52]; thus a more favorable surfactant/inhibitor ratio was obtained by giving a large amount of surfactant. Recently, Kobayashi and colleagues investigated the capability of surfactant mixed with edema fluid at several ratios to restore lung function in immature rabbit fetuses, as measured by tidal volume at preset insufflation pressures [5]. It was demonstrated that surfactant (25 mg/ml) mixed with edema fluid at a protein to lipid ratio (P/L ratio) of 2.2 was capable of restoring lung function, whereas surfactant mixed with edema fluid at P/L ratio of 11.2, was not. Surface tension properties of these mixtures demonstrated high minimal surface tensions at $P/L \geq 3.4$ and low surface tensions at $P/L \leq 1.8$. These results confirm the hypothesis of the competition mechanism between surfactant and proteins, although it cannot be ruled out that some direct interaction between surfactant and proteins occurs.

The lung lavage model, used in the present study, has proven useful for a variety of experimental purposes, including e.g. testing of different surfactant preparations and demonstrating that exogenous surfactant restores blood gases to normal [20-22]. In this model, the concentration of the surfactant preparation (which is more or less comparable with all other natural surfactant preparations) needed to restore blood gases over a 3

hour observation period was 50 mg/kg (Fig. 1B). To get the same results when surfactant was mixed with human plasma (4 ml/kg ≈ 280 mg plasma proteins/kg), six times as much surfactant (300 mg/kg) was necessary to restore and stabilize gas exchange (Fig. 1A). This means that approximately 1 mg surfactant phospholipids is needed to overcome the inhibitory effect of 1 mg plasma proteins. In these experiments citrated plasma was used to obtain full inhibitory capacity, including fibrinogen. However, the inhibitory components in diseased lungs probably consist not only of products derived from blood but also consist of, for example, specific proteases, inflammatory mediators, bacteria, membranes, etc. Plasma-derived proteins, however, may be the most important compounds of high-permeability edema fluid and thus, from this point of view, it makes sense to "titrate" the amount of surfactant for replacement therapy by mixing it with full plasma.

Our results favor the competition hypothesis: when mixed with plasma, surfactant at 50 mg/kg shows no improvement, at 100 mg/kg there is only transient improvement, and at 300 mg/kg there is sustained improvement of blood gas values. These results imply that for treatment of ARDS or established RDS a high concentration of surfactant is required to overcome the inhibitory effect of plasma-derived proteins. If after surfactant instillation there is no, or only transient, improvement of blood gas values in patients with either ARDS or RDS (fibrotic lungs excluded) this does not mean that surfactant treatment does not work. It only means that the concentration of the surfactant preparation used is too low in relation to the amount of surfactant inhibitors in the lungs. This raises the question of how to exclude the existence of fibrotic lungs. In our clinical practice we investigate this as follows: we increase the mean airway pressure in mechanically ventilated patients and observe blood gases. If blood gases improve this would mean that there are still recruitable lung parts which could be stabilized by exogenous surfactant treatment. On the other hand, if blood gases remain stable or even deteriorate after increasing mean airway pressure due to further mismatch of the ventilation/perfusion ratio, surfactant instillation may worsen the clinical situation by filling up the remaining areas participating in gas exchange, which is typical for fibrotic lungs. Thus, before giving surfactant, especially after a long period of artificial ventilation (2-3 weeks), one should always examine if there is still some lung tissue left which can be recruited by high ventilation pressures. As a consequence, in treatment of

ARDS or established RDS: 1) surfactant should be given as early as possible and 2) an excess of surfactant should be given, or repeatedly be substituted ("titrated") at low concentrations until blood gases improve.

REFERENCES

1. Ennema JJ, Kobayashi T, Robertson B, Curstedt T (1988) Inactivation of exogenous surfactant in experimental respiratory failure induced by hyperoxia. Acta Anaesthesiol Scand 32: 665-671
2. Ikegami M, Jacobs H, Jobe A (1983) Surfactant function in respiratory distress syndrome. J Pediatr 102: 443-447
3. Johnson JWC, Permutt S, Sipple JH, Salem ES (1964) Effect of intra-alveolar fluid on pulmonary surface tension properties. J Appl Physiol 19: 769-777
4. Kobayashi T, Ganzuka M, Tanigushi J, Nitta K, Murakami S (1990) Lung lavage and surfactant replacement for hydrochloric acid aspiration in rabbits. Acta Anaesthesiol Scand 34: 216-221
5. Kobayashi T, Nitta K, Ganzuka M, Inui S, Grossmann G, Robertson B (1991) Inactivation of exogenous surfactant by pulmonary edema fluid. Pediatr Res 29: 353-356
6. Said SI, Avery ME, Davis RK, Banerjee CM, El-Cohary M (1965) Pulmonary surface activity in induced pulmonary edema. J Clin Invest 44: 458-464
7. Seeger W, Stöhr G, Wolf HRD, Neuhof H (1985) Alteration of surfactant function due to protein leakage: special interaction with fibrin monomer. J Appl Physiol 58: 326-338
8. Guyton AC, Moffatt DS, Adair TH (1984) Role of alveolar surface tension in transepithelial movement of fluid. In: Robertson B, Van Golde LMG, Batenburg JJ (eds) Pulmonary surfactant. Elsevier Science Publishers, Amsterdam, pp 171-185
9. Fujiwara T, Chida S, Watabe Y, Maeta H, Morita T, Abe T (1980) Artificial surfactant therapy in Hyaline-Membrane Disease. Lancet 1: 55-59
10. Hallman M, Merritt TA, Jarvenpaa A-L, Boynton B, Mannino F, Gluck L, Moore T, Edwards D (1985) Exogenous human surfactant for treatment of severe respiratory distress syndrome: a randomized clinical trial. J Pediatrics 106: 963-969
11. Konishi M, Fujiwara T, Naito T, Takeuchi Y, Ogawa Y, Inukai K, Fujimura M, Nakamura H, Hashimoto T (1988) Surfactant replacement therapy in neonatal respiratory distress syndrome: A multi-centre, randomized clinical trial: comparison of high- versus low-dose of Surfactant TA. Eur J Pediatr 147: 20-25
12. Collaborative European Multicenter Study Group (1988) Surfactant replacement therapy for severe neonatal respiratory distress syndrome: an international randomized clinical trial. Pediatrics 82: 683-691
13. Ten Centre Study Group (1987) Ten centre trial of artificial surfactant (artificial lung expanding compound) in very premature babies. Br Med J 294: 991-996

14. Dunn MS, Shennan AT, Possmayer F (1990) Single- versus multiple-dose surfactant replacement therapy in neonates of 30 to 36 weeks' gestation with respiratory distress syndrome. Pediatrics 86: 564-571

15. Lachmann B (1987) The role of pulmonary surfactant in the pathogenesis and therapy of ARDS. In: Vincent JL (ed) Update in intensive care and emergency medicine. Springer-Verlag, Berlin, pp 123-134

16. Nosaka S, Sakai T, Yonekura M, Yoshikawa K (1990) Surfactant for adults with respiratory failure. Lancet 1: 947-948

17. Richman PS, Spragg RG, Robertson B, Merritt TA, Curstedt T (1989) The adult respiratory distress syndrome: first trials with surfactant replacement. Eur Respir J 2 (Suppl 3): 109s-111s

18. Marraro G (1991) Respiratory emergencies and supplementary surfactant in the treatment of severe RDS in leukaemic adolescents. In: Cosmi EV, Di Renzo GC, Anceschi MM (eds) The surfactant system of the lung: prevention and treatment of neonatal and adult respiratory distress syndrome. Macmillan Press, pp 198-206

19. Joka Th, Obertacke U (1989) Neue medikamentöse Behandlung im ARDS: Effekt einer intrabronchialen xenogenen Surfactantapplikation. Z Herz-, Thorax-,Gefäßchir 3 (Suppl 1): 21-24

20. Lachmann B, Robertson B, Vogel J (1980) In vivo lung lavage as an experimental model of the respiratory distress syndrome. Acta Anaesthesiol Scand 24: 231-236

21. Berggren P, Lachmann B, Curstedt T, Grossmann G, Robertson B (1986) Gas exchange and lung morphology after surfactant replacement in experimental adult respiratory distress syndrome induced by repeated lung lavage. Acta Anaesthesiol Scand 30: 321-328

22. Robertson B, Lachmann B (1988) Experimental evaluation of surfactants for replacement therapy. Exp Lung Res 14: 279-310

23. Lachmann B, Jonson B, Lindroth M, Robertson B (1982) Modes of artificial ventilation in severe respiratory distress syndrome. Lung function and morphology in rabbits after wash-out of alveolar surfactant. Crit Care Med 10: 724-732

24. Metcalfe IL, Enhorning G, Possmayer F (1980) Pulmonary surfactant-associated proteins: their role in the expression of surface activity. J Appl Physiol 49: 34-41

25. Van Daal GJ, So KL, Gommers D, Eijking EP, Fiévez RB, Sprenger MJ, Van Dam DW, Lachmann B (1991) Intratracheal surfactant administration restores gas exchange in experimental adult respiratory distress syndrome associated with viral pneumonia. Anesth Analg 72: 589-595

26. Eijking EP, Van Daal GJ, Tenbrinck R, Luijendijk A, Sluiters JF, Hannappel E, Lachmann B (1991) Effect of surfactant replacement on pneumocystis carinii pneumonia in rats. Intensive Care Med 17: 475-478

27. Van Daal GJ, Bos JAH, Eijking EP, Gommers D, Hannappel E, Lachmann B (1992) Surfactant replacement therapy improves pulmonary mechanics in end-stage influenza A pneumonia in mice. Am Rev Respir Dis 145: 859-863

28. Bos AP, Tibboel D, Hazebroek FWJ, Molenaar JC, Lachmann B, Gommers D (1991) Surfactant replacement therapy in high-risk congenital diaphragmatic hernia. Lancet 1: 1279

29. Robertson B, Berry D, Curstedt T, Grossmann G, Ikegami M, Jacobs H, Jobe A, Jones (1985). Leakage of protein in the immature rabbit lung; effect of surfactant replacement. Respir Physiol 61: 265-276

30. Hallman M, Spragg R, Harrell JH, Moser KM, Gluck L (1982) Evidence of lung function abnormality in respiratory failure. Study of bronchoalveolar lavage phospholipids, surface activity phospholipase activity, and plasma myoinositol. J Clin Invest 70: 673-683

31. Petty TL, Reiss OK, Paul GW, Silvers GW, Elkins ND (1977) Characteristics of pulmonary surfactant in adult respiratory distress syndrome associated with trauma and shock. Am Rev Respir Dis 115: 531-536

32. Pison U, Seeger W, Buchhorn R, Joka T, Brand M, Obertacke U, Neuhof H, Schmit-Neuerburg KP (1989) Surfactant abnormalities in patients with respiratory failure after multiple trauma. Am Rev Respir Dis 140: 1033-1039

33. Von Wichert P, Kohl FV (1977) Decreased dipalmitoyllecithin content found in lung specimens from patients with so-called shock-lung. Intensive Care Med 3: 27-30

34. Avery ME, Mead J (1959) Surface properties in relation to atelectasis and hyaline membrane disease. Am J Dis Child 97: 517-523

35. Ikegami M, Jobe A, Glatz T (1981) Surface activity following natural surfactant treatment of premature lambs. J Appl Physiol 51: 306-312

36. Ikegami M, Jobe A, Jacobs H, Lam R (1984) A protein from airways of premature lambs that inhibits surfactant function. J Appl Physiol 57: 1134-1142

37. Ikegami M, Jobe A, Berry D (1986) A protein that inhibits surfactant in respiratory distress syndrome. Biol Neonate 50: 121-129

38. Kobayashi T, Curstedt T, Grossmann G, Robertson B (1989) Inhibition of exogenous surfactant in ventilated immature newborn rabbits. Respir Physiol 76: 1-12

39. Tierney DF, Johnson RP (1965) Altered surface tension of lung extracts and lung mechanics. J Appl Physiol 20: 1253-1260

40. Fuchimukai T, Fujiwara T, Takahashi A, Enhorning G (1987) Artificial pulmonary surfactant inhibited by proteins. J Appl Physiol 62: 429-437

41. Holm BA, Notter RH, Finkelstein JN (1985) Surface property changes from interactions of albumin with natural lung surfactant and extracted lung lipids. Chem Phys Lipids 38: 287-298

42. Holm BA, Notter RH (1987) Effects of hemoglobin and cell membrane lipids on pulmonary surfactant activity. J Appl Physiol 63: 1434-1442

43. Holm BA, Enhorning G, Notter RH (1988) A biophysical mechanism by which plasma proteins inhibit lung surfactant activity. Chem Phys Lipids 49: 49-55

44. Keough KMW, Parsons CS, Phang PT, Tweedale MG (1988) Interactions between plasma proteins

and pulmonary surfactant: surface balance studies. Can J Physiol Pharmacol 66: 1166-1173

45. Taylor Jr. FB, Abrams ME (1966) Effect of surface active lipoprotein on clotting and fibrinolysis, and of fibrinogen on surface tension of surface active lipoprotein (with a hypothesis on the pathogenesis of pulmonary atelectasis and hyaline membrane in respiratory distress syndrome of the newborn). Am J Med 40: 346-350

46. Holm BA, Venkitaraman AR, Enhorning G, Notter RH (1990) Biophysical inhibition of synthetic lung surfactants. Chem Phys Lipids 52: 243-250

47. Ikegami M, Jobe A, Jacobs H, Jones S (1981) Sequential treatments of premature lambs with an artificial surfactant and natural surfactant. J Clin Invest 68: 491-496

48. Ikegami M, Agata Y, Elkady T, Hallman M, Berry D, Jobe A (1987) Comparison of four surfactants: in vitro surface properties and responses of preterm lambs to treatment at birth. Pediatrics 79: 38-46

49. Cockshutt AM, Weitz J, Possmayer F (1990) Pulmonary surfactant-associated protein A enhances the surface activity of lipid extract surfactant and reverses inhibition by blood proteins in vitro. Biochemistry 29: 8424-8429

50. Seeger W, Günther A, Thede C (1992) Differential sensitivity to fibrinogen inhibition of SP-C- vs. SP-B-based surfactants. Am J Physiol (Lung Cell Mol Physiol) 5: L286-L291

51. Balis JU, Shelley SA, McCue MJ, Rappaport ES (1971) Mechanisms of damage to the lung surfactant system. Ultrastructure and quantitation of normal and in vitro inactivated lung surfactant. Exp Mol Pathol 14: 243-262

52. Lachmann B, Hallman M, Bergmann KC (1987) Respiratory failure following anti-lung serum: study on mechanisms associated with surfactant system damage. Exp Lung Res 12: 163-180

CHAPTER 5

PREVENTION OF RESPIRATORY FAILURE AFTER HYDROCHLORIC ACID ASPIRATION BY INTRATRACHEAL SURFACTANT INSTILLATION IN RATS

E.P. Eijking, D. Gommers, K.L. So, M.P.M. de Maat[#],
J.W. Mouton* and B. Lachmann

Depts. of Anesthesiology, [#]Internal Medicine II and *Clinical Microbiology,
Erasmus University, Rotterdam, The Netherlands

In: Anesth Analg 1993; 76: 472-477

SUMMARY

As the surfactant system is probably involved in the pathophysiology of respiratory failure caused by hydrochloric acid (HCl) aspiration, a study was designed to investigate the effects of different ventilation strategies and intratracheal surfactant instillation at different time intervals, on the course of pulmonary gas exchange after HCl aspiration in rats. In this study rats were anesthetized, tracheotomized and mechanically ventilated. Respiratory failure was induced by intratracheal instillation of 3 ml/kg 0.1 N HCl. Animals (n=49) were divided into 9 groups: Groups 1 and 2-9 were ventilated with peak airway pressure/PEEP of 14/2 and 26/6 cmH_2O, respectively; Groups 3 and 4 received surfactant (200 mg/kg) intratracheally, 1 and 10 min after HCl aspiration; Groups 5 and 6 received saline, 1 and 10 min after HCl aspiration; Groups 7 and 8 received surfactant, 60 and 90 min after HCl aspiration; Group 9 received saline instead of HCl. Gas exchange deteriorated in Groups 1, 2, 5, 6, 7 and 8, whereas respiratory failure could be prevented in Groups 3 and 4. After deterioration of gas exchange, surfactant treatment prevented further decrease of PaO_2 values in Group 7, whereas no effect on gas exchange was observed in Group 8; intratracheal instillation of saline had no effect on gas exchange (Group 9). These results suggest that surfactant should be given as early as possible after aspiration of gastric contents, to prevent development of respiratory failure.

INTRODUCTION

Massive aspiration of gastric contents is one of the most feared complications of general anesthesia and is an important cause of the adult respiratory distress syndrome (ARDS) (1, 2). ARDS caused by aspiration of gastric contents is characterized by deterioration of gas exchange requiring mechanical ventilation with high inspiratory oxygen concentration, high insufflation pressure and positive end-expiratory pressure (PEEP), and is associated with a mortality rate of over 90% (2). Hydrochloric acid (HCl) causes direct damage to the alveolar-capillary membrane, leading to influx of protein-rich edema fluid into the alveolar space (3-6). These plasma-derived proteins are known to be potent inhibitors of surfactant function (7-12). It has also been proposed that HCl

directly damages the surfactant system (4, 13).

As the surfactant system is probably involved in the pathophysiology of respiratory failure caused by HCl aspiration, studies have been performed to investigate the effect of surfactant replacement therapy on lung function of animals suffering from respiratory failure due to HCl aspiration. Kobayashi and colleagues demonstrated that surfactant instillation could only partly restore gas exchange in rabbits suffering from respiratory failure due to HCl aspiration after lung edema was removed by bronchoalveolar lavage; surfactant, when given without prior lung lavage, served only to prevent further deterioration of blood gases (6). Lamm and colleagues showed improved lung recoil without improvement in gas exchange in rabbits receiving surfactant 5 min after HCl aspiration (14).

The aim of this study was to investigate the effects of different ventilation strategies and intratracheal surfactant instillation at different time intervals, on the course of pulmonary gas exchange after HCl aspiration in rats.

MATERIALS AND METHODS

Exogenous surfactant

The surfactant used in these experiments is a freeze-dried natural surfactant isolated from bovine lungs in basically the same manner as previously described (15). It consists of approximately 90% phospholipids, 1% hydrophobic proteins (SP-B and SP-C), the remainder being other lipids such as cholesterol, glyceride and free fatty acids. There is no SP-A (the largest surfactant associated protein, MW: 26-38 kDa) in this surfactant preparation as a result of isolation procedures. Surfactant was suspended in saline at a concentration of 50 mg dry weight/ml. This surfactant preparation has proven to be highly effective in improving gas exchange and lung mechanics in various animal models of respiratory failure of differing etiologies (16-18).

Animal study

The study protocol was approved by the Animal Care Committee of Erasmus University Rotterdam, The Netherlands.

The studies were performed in 49 male adult Sprague-Dawley rats (body weight:

300-350 g). After induction of anesthesia with nitrous oxide, oxygen and halothane (65/33/2%) the animals were tracheotomized and a catheter was inserted into the carotid artery. Anesthesia was maintained with pentobarbital sodium (60 mg/kg, i.p.) and muscle relaxation was attained with pancuronium bromide (0.5 mg/kg, i.m.). Lungs were ventilated with a Servo Ventilator 900 C (Siemens-Elema, Solna, Sweden) at the following ventilator settings: pressure-controlled ventilation, $F_iO_2=1.0$, ventilation frequency=30/min, peak airway pressure (P_{peak}) =14 cmH_2O, PEEP =2 cmH_2O and inspiratory/expiratory ratio=1:2. After reaching steady state (PaO_2> 500 mmHg), 43 rats received 1.5 ml/kg HCl intratracheally (0.1 N) while lying on their right side, followed by a bolus of air (30 ml/kg). This was immediately followed by instillation of 1.5 ml/kg HCl, while lying on their left side, again followed by a bolus of air. Six rats received saline (2 x 1.5 ml/kg) instead of HCl.

Directly after instillation, P_{peak} was increased to 26 cmH_2O and PEEP to 6 cmH_2O in 38 rats receiving HCl and in the 6 rats receiving saline. P_{peak} was kept at 14 cmH_2O and PEEP at 2 cmH_2O in 5 rats receiving HCl. In all animals the same ventilator settings were maintained throughout the observation period.

The animals were divided into 9 groups (Table 1): Group 1 (n=5) was ventilated at P_{peak}=14 cmH_2O and PEEP=2 cmH_2O (HCl 14/2); Group 2 (n=6) was ventilated at P_{peak}=26 cmH_2O and PEEP=6 cmH_2O (HCl 26/6); Group 3 (n=6) received surfactant intratracheally 1 min after HCl (Surf 1'); Group 4 (n=5) received surfactant 10 min after HCl (Surf 10'); Groups 5 and 6 (n=5 and n=5) received saline 1 and 10 min after HCl (Sal 1' and Sal 10', respectively); Group 7 (n=6) received surfactant 60 min after HCl (Surf 60') and Group 8 (n=5) received surfactant after 90 min (Surf 90'); Group 9 (n=6) received saline instead of HCl and served as control (NaCl). Animals from Groups 3, 4, 7 and 8 were treated with surfactant at a dose of 200 mg dry weight/kg body weight.

Blood samples for measurement of PaO_2 and $PaCO_2$ (ABL 330; Radiometer, Copenhagen, Denmark) were taken from the carotid artery of each animal before intratracheal instillation and at 15, 30, 60, 90 and 120 min post-instillation.

At the end of the experiments all animals were sacrificed with an overdose of intra-arterially administered pentobarbital sodium. Lungs of a few animals of Groups 2-6 and 9 underwent bronchoalveolar lavage (BAL) with saline (37°C; 30 ml/kg).

Table 1: Different treatment groups.

Group		n	HCl	P_{peak}/PEEP	Surf/Sal
1	(HCl 14/2)	5	+	14/2	-
2	(HCl 26/6)	6	+	26/6	-
3	(Surf 1')	6	+	26/6	Surf 1'
4	(Surf 10')	5	+	26/6	Surf 10'
5	(Sal 1')	5	+	26/6	Sal 1'
6	(Sal 10')	5	+	26/6	Sal 10'
7	(Surf 60')	6	+	26/6	Surf 60'
8	(Surf 90')	5	+	26/6	Surf 90'
9	(NaCl)	6	- (NaCl)	26/6	-

For explanation of abbreviations: see text

Bronchoalveolar lavage fluid

To compare all BAL samples with those from healthy controls, 6 additional male Sprague-Dawley rats (body weight 300-350 g) were lavaged once with saline (37°C; 30 ml/kg). The BAL fluids of the different groups were prepared as follows: all samples were centrifugated for 15 min at 2000 g to remove cell material. Protein concentration was measured in all samples using a modified Lowry method (19), with bovine serum albumin as standard. Also, surface activity in BAL fluid was measured using a modified Wilhelmy balance (E. Biegler GmbH, Mauerbach, Austria). In this method, a tight-fitting teflon barrier reduces the surface area of a teflon trough from 100-20% at a cycle speed of 0.33/min. Saline is used as subphase and is kept at 37°C. The force on a platinum slide (1x1 cm), dipped into the subphase, is measured by a force transducer and expressed as surface tension. Further, maximal surface tension is measured at 100% surface area and minimal surface tension at 80% surface compression and expressed as milli Newton/meter (mN/m). Surface tension characteristics of a BAL sample are

measured after application on the surface of the saline-filled trough. In this study 500 μl of BAL fluid was applied to the surface of the trough; maximal and minimal surface tensions were measured after 3 cycles.

Statistical analysis

All data are expressed as mean ± standard deviation (SD). For the animal study statistical analysis of data was performed using repeated measurements Anovas (20), with time as the repeat variable. Three Anovas were performed initially: the effect of P_{peak}/PEEP settings against controls; the effect of saline instillation against controls; and finally the effect of surfactant instillation against controls. When significant differences between groups occurred, the difference between these groups was further analyzed. For analysis of BAL parameters, standard Anova procedures were performed. Statistical significance was accepted at $p \leq 0.05$.

RESULTS

Animal study

Figure 1 shows PaO_2 values for each group. Before instillation of HCl or saline, PaO_2 values are high and there is no significant intergroup difference. There is a significant difference in PaO_2 values between rats ventilated at 14/2 or at 26/6 cmH_2O after HCl instillation (HCl 14/2 and HCl 26/6, respectively); saline (NaCl), instead of HCl, at the applied ventilator settings had no influence on PaO_2 values (Fig. 1A). There is no intergroup difference in PaO_2 values between rats ventilated at 26/6 cmH_2O, rats treated with saline at 1 min (Sal 1') and rats treated with saline at 10 min (Sal 10') after HCl instillation; for this reason PaO_2 values of these three groups are used (Control) for comparison with other treatment groups. The differences in PaO_2 values between rats treated with surfactant 1 or 10 min after HCl instillation (Surf 1' and Surf 10', respectively) and Control are significant; there is no intergroup difference between PaO_2 values of Surf 1' and Surf 10' (Fig. 1B). Although there is a significant difference in PaO_2 values prior to surfactant treatment between groups of rats treated at both 60 and 90 min after HCl instillation (Surf 60' and Surf 90', respectively) and Control, PaO_2 values

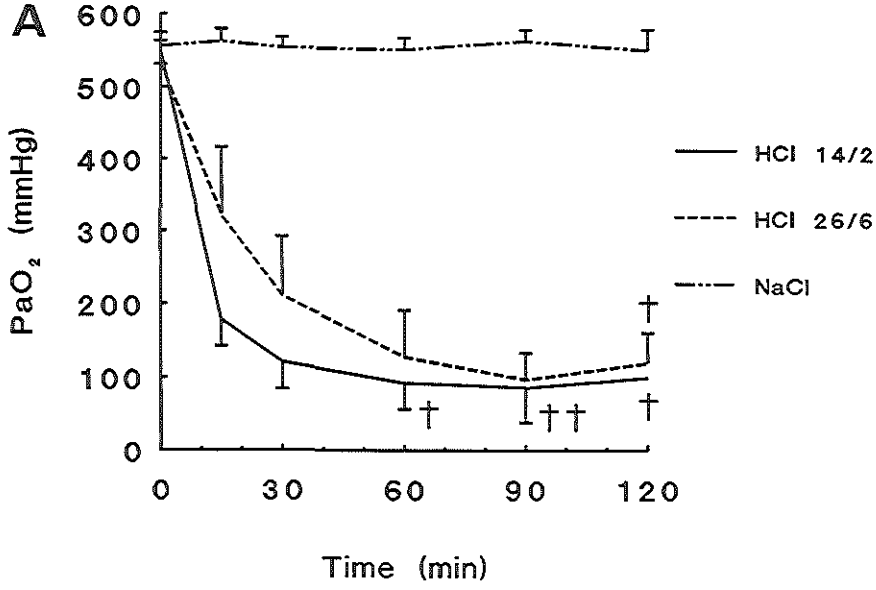
increase significantly compared to pre-treatment values in Surf 60', whereas there is no increase in PaO_2 values observed in Surf 90' (Fig. 1C).

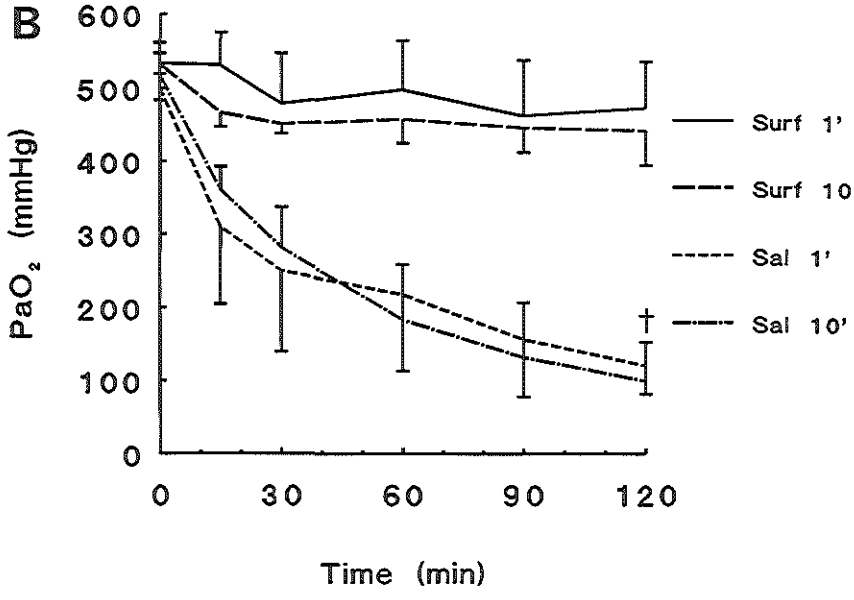
Figure 2 shows the $PaCO_2$ values for each group. Before instillation of HCl or saline there are no significant differences in $PaCO_2$ values between the groups. There is a significantly higher increase in $PaCO_2$ values in all animals ventilated at 14/2 cmH_2O compared to rats ventilated at 26/6 cmH_2O; in rats receiving saline instead of HCl, $PaCO_2$ values remained in physiological range (Fig. 2A). Again, there is no intergroup difference in $PaCO_2$ values between rats ventilated at 26/6 cmH_2O, rats treated with saline at 1 min and rats treated with saline at 10 min after HCl instillation and thus $PaCO_2$ values of these three groups are used (Control) for comparison with other treatment groups. Although $PaCO_2$ values of rats treated with surfactant at 1 or 10 min after HCl instillation appear to be lower compared to Control, these differences are not significant (Fig. 2B). There also is no significant difference in $PaCO_2$ values between rats receiving surfactant after 60 min and 90 min and Control (Fig. 2C).

Bronchoalveolar lavage fluid

Table 2 shows the recovery percentage, protein concentration and surface tension properties of BAL fluid samples. For statistical reasons the BAL parameters of rats receiving saline 1 and 10 min after HCl instillation were regarded to be comparable (as evidenced by a small SD), as were BAL parameters of rats receiving surfactant 1 and 10 min after HCl instillation.

The protein concentration in BAL fluid of animals receiving HCl is significantly higher than that of BAL fluid of controls and rats receiving saline instead of HCl; there is no significant difference in protein concentration between groups receiving HCl. Surface tension measurements demonstrate decrease in surface activity in BAL fluid from rats after HCl aspiration, not receiving surfactant treatment. BAL fluid from rats receiving surfactant 1 and 10 min after HCl aspiration demonstrate favorable surface activity (for detailed statistical differences between groups, see Table 2).


A
600
500
400
300
200
100
0
PaO2 (mmHg)
0
30
60
90
120
Time (min)
HCl 14/2
HCl 26/6
NaCl


B
600
500
400
300
200
100
0
PaO2 (mmHg)
0
30
60
90
120
Time (min)
Surf 1'
Surf 10'
Sal 1'
Sal 10'

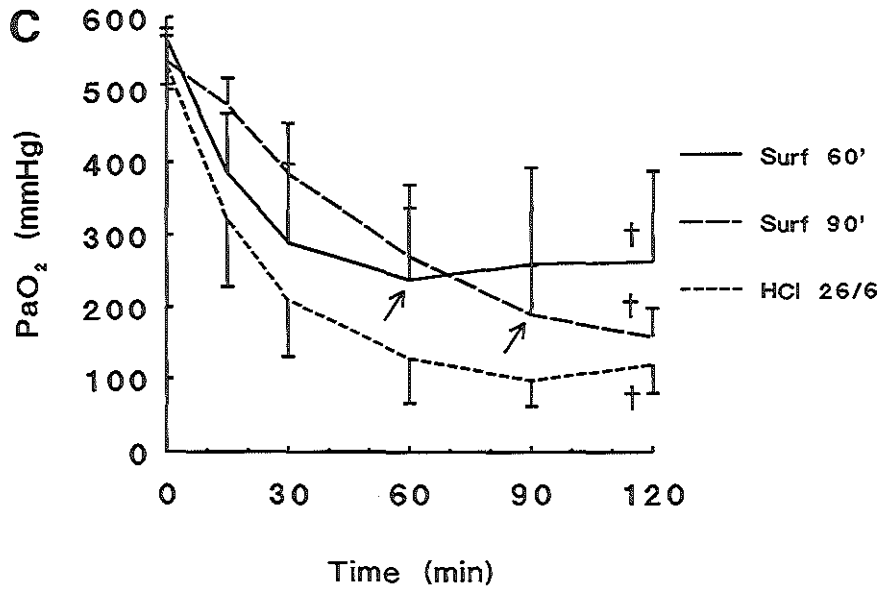
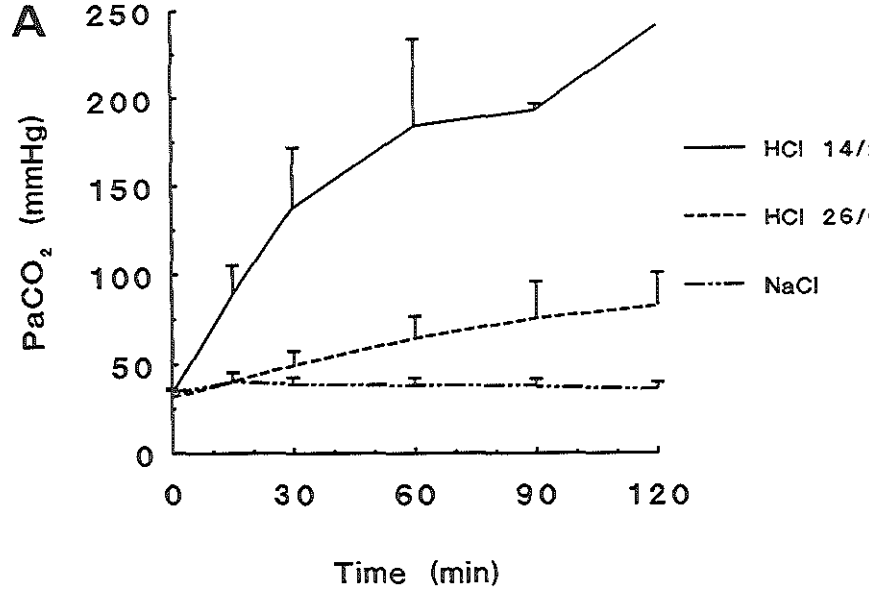


Figure 1. PaO_2 values (mean ± SD) of the different groups: rats ventilated at P_{peak}=14/PEEP=2 cmH_2O or P_{peak}=26/PEEP=6 cmH_2O after HCl aspiration (HCl 14/2 and HCl 26/6, respectively) and rats receiving saline (NaCl) instead of HCl (A); rats receiving surfactant or saline, 1 or 10 min after HCl aspiration (Surf 1', Surf 10', Sal 1' and Sal 10', respectively) (B); rats receiving surfactant 60 or 90 min after HCl aspiration (Surf 60' and Surf 90', respectively) and, again, rats ventilated at P_{peak}=26/PEEP=6 cmH_2O (HCl 26/6) (C); † = one rat died; ↑ = surfactant instillation; PaO_2= arterial oxygen tension.



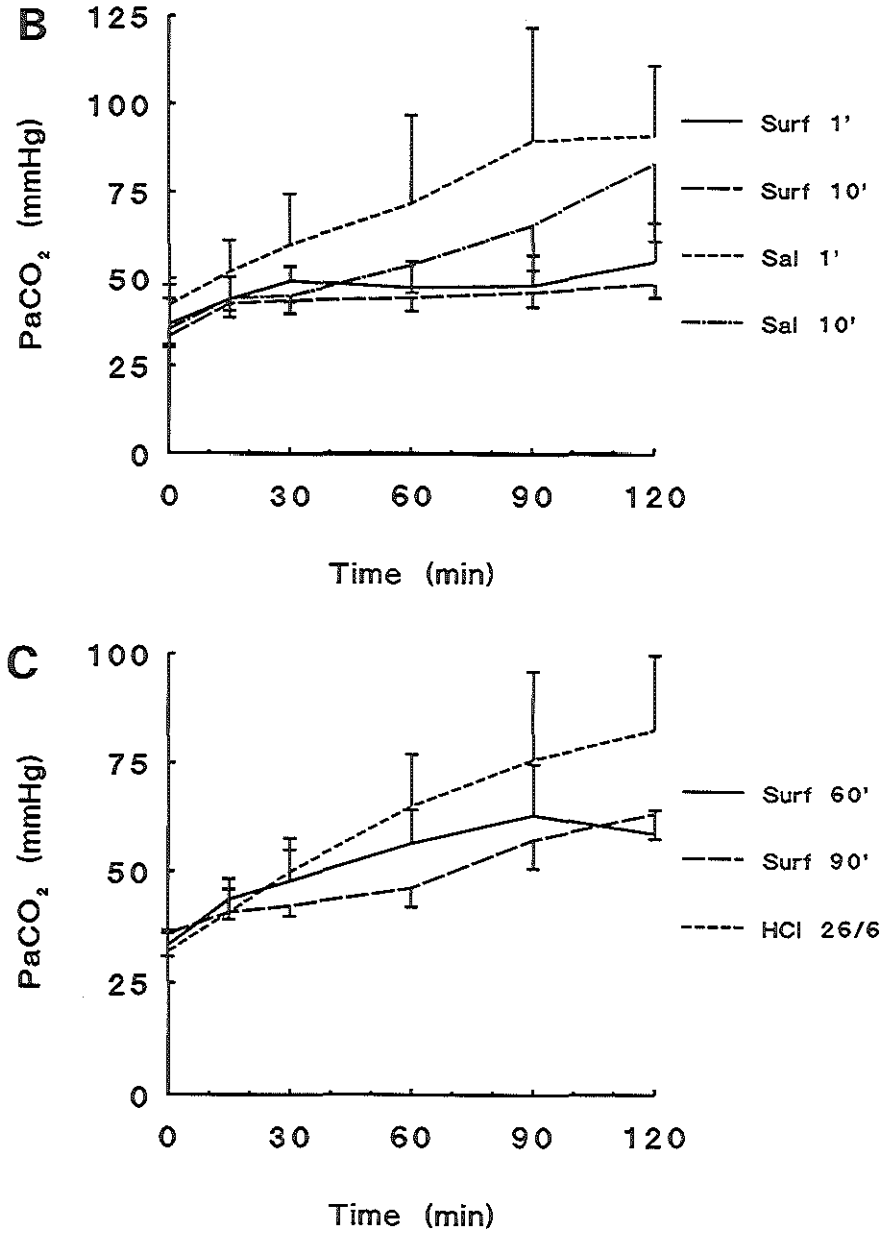


Figure 2. $PaCO_2$ values (mean ± SD) of the different groups; for explanation of abbreviations, see Figure 1; $PaCO_2$= arterial carbon dioxide tension.

Table 2: BAL parameters.

Sample	n	Recovery [%]	Protein [mg/ml]	Surface tension [mN/m]	
				maximal	minimal
Control	6	95.1 ± 5.23	0.24* ± 0.06	64.5 ± 5.83	24.2@ ± 3.99
Saline	5	93.8 ± 5.45	0.41* ± 0.42	62.9 ± 3.11	25.1§ ± 2.51
HCl (26/6)	5	98.1 ± 5.56	7.45 ± 1.33	60.5 ± 3.24	31.2 ± 2.33
HCl (Sal 1' + 10')	6	100.7 ± 5.62	8.07 ± 1.16	64.6 ± 4.56	34.3 ± 4.42
HCl (Surf 1' + 10')	7	95.5 ± 4.33	6.98 ± 1.31	46.8# ± 6.52	19.6¶ ± 4.11

All data are mean ± SD; Control= BAL fluid of healthy contol rats; Saline= BAL fluid of rats receiving saline instead of HCl, intratracheally; (26/6)= rats ventilated at P_{peak}=26 and PEEP=6 cmH_2O; (Sal 1' + 10') and (Surf 1' + 10')= rats receiving saline or surfactant, respectively, 1 or 10 min after HCl aspiration; *=significant difference between Control/Saline and other groups; #=significant difference between HCl (Surf 1' + 10') and other groups; @=significant difference between Control and both HCl (26/6) and HCl (Sal 1' + 10'); §=significant difference between Saline and all HCl groups; ¶=significant difference between HCl (Surf 1' + 10') and the two other HCl groups.

DISCUSSION

This study demonstrates that surfactant, when given within 10 min, prevents deterioration of gas exchange after massive aspiration of HCl (pH=1.0), but cannot prevent accumulation of plasma-derived proteins into the alveolar space. It is also shown that respiratory failure cannot be prevented by increasing insufflation pressure and PEEP to levels used in this study, although more animals survived during the whole observation period (HCl 14/2 versus HCl 26/6). Similar results have been reported by others; namely, that lungs of rabbits receiving surfactant (100 mg/kg body weight) 5 min after

HCl aspiration (pH = 1.0, 2 ml/kg) showed improved lung recoil compared to lungs which did not receive surfactant; however, this study did not demonstrate higher PaO_2 values in rabbits treated with surfactant, although it must be noted that low PEEP levels were used (14).

The mechanism of respiratory failure in HCl aspiration can be explained by a) damage to the alveolar-capillary membrane (4), leading to increased permeability (5, 21, 22) and thus influx of protein-rich edema fluid into the alveolar space; b) direct damage to the surfactant system (4, 13) and c) secondary inhibition of remaining surfactant activity caused by plasma-derived proteins present in the edema fluid (7-12). As a result of (functional) surfactant deficiency, suction force across the alveolar-capillary membrane increases, leading to further outpouring of edema fluid into the alveolar space: this way a vicious circle exists. Also, as a result of diminished surfactant function, surface tension on the alveolar wall increases, leading to increased retractive forces across the alveolar-capillary membrane, with subsequent formation of atelectasis and mismatch of the ventilation-perfusion ratio, leading to hypoxemia.

Surfactant is inhibited by plasma-derived proteins in a dose-dependent manner (7-12). Thus, with a high surfactant/inhibitor (S/I) ratio there is no inhibition of surfactant function, whereas with a low S/I ratio most surfactant function is abolished. Although the exact mechanism of inhibition remains unclear, it seems that competition between surfactant and inhibitors at the air-liquid interphase of the alveolar wall plays an important role (9, 12). When surfactant is given before deterioration of gas exchange (i.e. before the remaining surfactant function is inhibited) the S/I ratio is increased, thus preventing the pathophysiological mechanisms that lead to respiratory failure. It is emphasized that the initial damage to lung tissue by HCl cannot be prevented, as HCl is neutralized within 30 seconds (21) and, thus, lung structure is already damaged. This is confirmed in the present study, where protein concentration in BAL fluid of rats receiving surfactant within 10 min is almost as high as protein concentration of rats receiving saline, or ventilated only. Comparable findings were also reported in the study mentioned earlier (14), namely increased wet/dry weight ratio in lungs of rabbits which received surfactant 5 min after HCl aspiration as well as in lungs of rabbits not receiving surfactant. However, surface tension properties of BAL fluid of rats receiving surfactant demonstrated low minimal and low maximal surface tensions compared to BAL fluid of

rats receiving saline. Thus, due to the lower surface tension of the "intrapulmonary fluid" the applied ventilator settings (26/6 cmH_2O) were sufficient for maintaining (normal) gas exchange.

Earlier studies have demonstrated that intratracheal surfactant instillation fully restores lung function in animals suffering from respiratory failure due to pneumonia (16-18). Also, gas exchange in rats suffering from respiratory failure after bilateral whole lung lavage could be fully restored after surfactant treatment (23). In the present study surfactant treatment, 60 min after HCl instillation, showed slight improvement of gas exchange; surfactant treatment 90 min after HCl instillation did not prevent further deterioration of gas exchange during the observation period. A possible explanation could be that after HCl aspiration lung structure is more severely damaged compared with animals suffering from pneumonia, or after whole lung lavage. This would lead to increased permeability of the alveolar-capillary membrane and more severe intra-alveolar edema accumulation. After deterioration of gas exchange due to HCl aspiration, S/I ratio (before surfactant instillation) is low and lung structure is damaged. When surfactant is given in these conditions, little or no effect is seen on gas exchange. A possible explanation is that surfactant, at a concentration used in this study (200 mg/kg), is directly inhibited by high concentration of inhibitors. Another explanation could be that the edema fluid and atelectatic areas form a mechanical barrier, preventing surfactant from entering the alveolar spaces. However, there are studies in which gas exchange could be improved either by giving a large amount of surfactant (280-350 mg/kg body weight) (24), or by lavaging the lungs with saline before surfactant instillation (6), as already mentioned in the introduction. With both methods an improved S/I ratio could be achieved.

Similar results have also been reported in animals suffering from respiratory failure due to hyperoxic lung injury. Surfactant substitution (60 mg/kg body weight) can prevent development of respiratory insufficiency in rabbits exposed to 100% oxygen for 64 hours: PaO_2 values of surfactant-treated rabbits, during mechanical ventilation with 100% oxygen, remained at high levels compared with saline-treated animals (25). Also, wet/dry ratio in animals receiving surfactant was high compared to lungs of healthy controls. However, in guinea pigs suffering already from respiratory failure due to prolonged exposure to 100% oxygen, surfactant treatment (200 mg/kg body weight) was

not able to restore gas exchange (7).

In the present study a "point of no return" mechanism seems to be applicable to the situation: after deterioration of gas exchange, little or no beneficial effect can be seen after surfactant instillation at a concentration used in this study (200 mg/kg body weight), whereas gas exchange is preserved when surfactant is given within 10 min. Although it is difficult to extrapolate from this animal study to the clinical situation, our results suggest that when massive aspiration of gastric contents is suspected, surfactant should be given as soon as possible to prevent development of respiratory failure. An important consideration which favors direct treatment with surfactant is that ARDS, caused by aspiration of gastric contents, has a mortality rate of over 90% (2).

In conclusion: increasing insufflation pressure and PEEP alone to levels used in this study has no beneficial effect on the course of hypoxemia after HCl aspiration in rats. Surfactant given within 10 min after HCl aspiration does prevent development of respiratory failure but does not prevent damage to lung structure, as evidenced by high protein concentrations in BAL fluid. Surfactant, at the dose used in this study, given after deterioration of gas exchange, can only prevent further decrease in PaO_2 values.

REFERENCES

1. Gibbs CP, Modell JH. Management of aspiration pneumonitis. In: Miller RD, ed. Anesthesia. New York, Edinburgh, London, Melbourne: Churchill Livingstone, 1990: 1293-319.
2. Fowler AA, Hamman RF, Good JT, et al. Adult respiratory distress syndrome: risk with common predispositions. Ann Intern Med 1983; 98: 593-7.
3. Awe WC, Fletcher WS, Jacob SW. The pathophysiology of aspiration pneumonitis. Surgery 1966; 60: 232-9.
4. Greenfield LJ, Singleton RP, McCaffree DR, Coalson JJ. Pulmonary effects of experimental graded aspiration of hydrochloric acid. Ann Surg 1969; 170: 74-86.
5. Grimbert FA, Parker JC, Taylor AE. Increased pulmonary vascular permeability following acid aspiration. J Appl Physiol 1981; 51: 335-45.
6. Kobayashi T, Ganzuka M, Tanigushi J, Nitta K, Murakami S. Lung lavage and surfactant replacement for hydrochloric acid aspiration in rabbits. Acta Anaesthesiol Scand 1990; 34: 216-21.
7. Ennema JJ, Kobayashi T, Robertson B, Curstedt T. Inactivation of exogenous surfactant in experimental respiratory failure induced by hyperoxia. Acta Anaesthesiol Scand 1988; 32: 665-71.
8. Fuchimukai T, Fujiwara T, Takahashi A, Enhorning G. Artificial pulmonary surfactant inhibited by

proteins. J Appl Physiol 1987; 62: 429-37.

9. Holm BA, Enhorning G, Notter RH. A biophysical mechanism by which plasma proteins inhibit lung surfactant activity. Chem Phys Lipids 1988; 49: 49-55.

10. Ikegami M, Jobe A, Jacobs H, Lam R. A protein from airways of premature lambs that inhibits surfactant function. J Appl Physiol 1984; 57: 1134-42.

11. Seeger W, Stöhr G, Wolf HRD, Neuhof H. Alteration of surfactant function due to protein leakage: special interaction with fibrin monomer. J Appl Physiol 1985; 58: 326-38.

12. Holm BA, Notter RH. Effects of hemoglobin and cell membrane lipids on pulmonary surfactant activity. J Appl Physiol 1987; 63: 1434-42.

13. Winn R, Stothert J, Nadir B, Hildebrandt J. Lung Mechanics following aspiration of 0.1 N hydrochloric acid. J Appl Physiol 1983; 55: 1051-6.

14. Lamm WJE, Albert RK. Surfactant replacement improves lung recoil in rabbit lungs after acid aspiration. Am Rev Respir Dis 1990; 142: 1279-83.

15. Metcalfe IL, Enhorning G, Possmayer F. Pulmonary surfactant-associated proteins: their role in the expression of surface activity. J Appl Physiol 1980; 49: 34-41.

16. Van Daal GJ, So KL, Gommers D, et al. Intratracheal surfactant administration restores gas exchange in experimental adult respiratory distress syndrome associated with viral pneumonia. Anesth Analg 1991; 72: 589-95.

17. Eijking EP, Van Daal GJ, Tenbrinck R, et al. Effect of surfactant replacement on pneumocystis carinii pneumonia in rats. Intensive Care Med 1991; 17: 475-8.

18. Van Daal GJ, Bos JAH, Eijking EP, Gommers D, Hannappel E, Lachmann B. Surfactant replacement therapy improves pulmonary mechanics in end-stage influenza A pneumonia in mice. Am Rev Respir Dis 1992; 145: 859-63.

19. Markwell MAK, Haas SM, Bieber LL, Tolbert NE. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem 1978; 87: 206-10.

20. SAS Users Guide. SAS Institute Inc., Cary, N.C. (U.S.A.), 1989.

21. Jones JG, Berry M, Hulands GH, Crawley JCW. The time course and degree of change in alveolar-capillary membrane permeability induced by aspiration of hydrochloric acid and hypotonic saline. Am Rev Respir Dis 1978; 118: 1007-13.

22. Kennedy TP, Johnson KJ, Kunkel RG, Ward PA, Knight PR, Finch JS. Acute acid aspiration lung injury in the rat: biphasic pathogenesis. Anesth Analg 1989; 69: 87-92.

23. Berggren P, Lachmann B, Curstedt T, Grossmann G, Robertson B. Gas exchange and lung morphology after surfactant replacement in experimental adult respiratory distress syndrome induced by repeated lung lavage. Acta Anaesthesiol Scand 1986; 30: 321-8.

24. Lachmann B, Hallman M, Bergmann KC. Respiratory failure following anti-lung serum: study on mechanisms associated with surfactant system damage. Exp Lung Res 1987; 12: 163-80.

25. Matalon S, Holm BA, Notter RH. Mitigation of pulmonary hyperoxic injury by administration of exogenous surfactant. J Appl Physiol 1987; 62: 756-61.

CHAPTER 6

SURFACTANT TREATMENT OF RESPIRATORY FAILURE INDUCED BY HYDROCHLORIC ACID ASPIRATION IN RATS

E.P. Eijking, D. Gommers, K.L. So, M. Vergeer and B. Lachmann

Dept. of Anesthesiology, Erasmus University,
Rotterdam, The Netherlands

In: Anesthesiology 1993 (in press)

This topic was presented at the 6th European Congress on Intensive Care Medicine in Barcelona in October 1992. An award was received for this presentation.

SUMMARY

Background: The surfactant system seems to be involved in the pathophysiology of respiratory failure caused by hydrochloric acid (HCl) aspiration. This study investigated the effect of different treatment strategies using an exogenous surfactant preparation on lung function of rats suffering from respiratory failure after intratracheal HCl instillation.
Methods: In rats anesthetized with halothane, nitrous oxide and oxygen, tracheotomy was performed and the lungs were mechanically ventilated. Respiratory failure was induced by intratracheal instillation of HCl (0.1 N, 3 ml/kg). After the PaO_2 decreased to <200 mmHg, the animals were randomly divided into 5 groups: Group I received no treatment; Group II received a natural surfactant preparation intratracheally (200 mg/kg); Group III underwent bronchoalveolar lavage (BAL) with saline, followed by surfactant treatment (200 mg/kg); Groups IV and V underwent BAL with saline and a diluted surfactant suspension (3.3 mg/ml in 30 ml/kg), respectively. Groups IV and V received a second and a third BAL 60 and 120 min after the first lavage. Blood gas analysis and protein measurements in BAL fluids were performed.
Results: Gas exchange improved in Groups III and V only. Protein concentrations were high in all BAL fluids. In the rats receiving BAL three times (Groups IV and V), a decrease in protein concentration was observed.
Conclusions: From these results it is concluded that surfactant proteins (which are known to inhibit surfactant function) are washed out of the alveoli, resulting in improved efficacy of surfactant treatment.

INTRODUCTION

Massive aspiration of gastric contents is one of the most feared complications of general anesthesia and is an important cause of the adult respiratory distress syndrome (ARDS) (for review, see [1,2]). ARDS caused by aspiration of gastric contents is characterized by deterioration of gas exchange requiring mechanical ventilation with high inspiratory oxygen concentration, high inspiratory pressure and positive end-expiratory pressure (PEEP), and is associated with a mortality rate of over 90% (2). Hydrochloric acid (HCl) causes direct damage to the alveolar-capillary membrane, leading to influx of protein-rich

edema fluid into the alveolar space (3-6). These plasma-derived proteins are known to be potent inhibitors of pulmonary surfactant function (7-13).

Several studies have been performed in which animals suffering from respiratory failure due to HCl aspiration were treated with surfactant. Kobayashi et al (6) demonstrated that surfactant instillation could only partly restore gas exchange in rabbits suffering from respiratory failure due to HCl aspiration after intra-alveolar lung edema was removed by bronchoalveolar lavage (BAL) with saline. Lamm et al (14) showed improved lung recoil in rabbits receiving surfactant 5 min after HCl aspiration, but no effect was seen on blood gases. Strohmaier et al (15), in a study on rabbits suffering from respiratory failure due to HCl aspiration, also were unable to improve oxygenation after surfactant treatment. In a recent study using this model we reported that surfactant, when given as a bolus at 200 mg/kg body weight after development of respiratory failure, was only able to prevent further deterioration of lung function, but was unable to improve gas exchange (16). For this reason the present study was designed to investigate whether there is any possibility to improve blood gases to almost normal values using an exogenous surfactant preparation in rats suffering from respiratory failure due to HCl aspiration. Different surfactant substitution regimes are investigated; the removal of edema fluid from the alveolar space with a diluted surfactant suspension was of particular interest.

The surfactant preparation used in this study has proven to be highly effective in improving gas exchange and lung mechanics in various animal models of respiratory failure of differing etiologies (17-19) and in newborns suffering from respiratory failure due to congenital diaphragmatic hernia (20).

MATERIALS AND METHODS

Exogenous surfactant

The surfactant used in these experiments is a freeze-dried natural surfactant isolated from bovine lungs in basically the same manner as previously described (21). It consists of approximately 90% phospholipids, 1% hydrophobic surfactant-associated proteins (so called SP-B and SP-C), the remainder being other lipids such as cholesterol, glyceride and free fatty acids. There is no SP-A (the largest surfactant-associated protein,

molecular weight: 26-38 kDa) in this surfactant preparation. Surfactant was suspended in saline.

Animal study

This protocol was approved by the Animal Care and Use Committee of the Erasmus University Rotterdam, the Netherlands.

The studies were performed in 44 male adult Sprague-Dawley rats (body weight: 300-350 g). After induction of anesthesia with nitrous oxide, oxygen and halothane (65/33/2%) a tracheotomy was performed and a catheter was inserted into the carotid artery. Anesthesia was maintained with pentobarbital sodium (60 mg/kg/h, i.p.) and muscle relaxation was attained with pancuronium bromide (0.5 mg/kg/h, i.m.). The lungs were ventilated with a Servo Ventilator 900 C (Siemens-Elema, Solna, Sweden) at the following ventilator settings: pressure-controlled ventilation, $F_iO_2 = 1.0$, ventilation frequency=30/min, peak airway pressure (P_{peak})=14 cmH_2O, PEEP=2 cmH_2O and inspiratory/expiratory ratio = 1:2. After reaching steady state ($PaO_2 > 500$ mmHg), 38 rats received 1.5 ml/kg HCl intratracheally (0.1 N; pH=1.0) while lying on their right side, followed by a bolus of air (30 ml/kg). This was immediately followed by instillation of 1.5 ml/kg HCl, while lying on their left side, again followed by a bolus of air; 6 rats received saline (2x1.5 ml/kg) instead of HCl and served as controls. Directly after instillation, P_{peak} was increased to 26 cmH_2O and PEEP to 6 cmH_2O in all rats; these ventilator settings were maintained throughout the whole observation period.

After PaO_2 decreased to <200 mmHg (within 1-2 h) in rats receiving HCl, the animals were randomly divided into 5 groups: Group I (n=7) received no treatment; Group II (n=8) received surfactant intratracheally; in Group III (n=7) the lungs were lavaged with saline followed by intratracheal surfactant instillation; in Groups IV and V (n=8 per group) the lungs were lavaged with saline or a diluted surfactant suspension, respectively, at two time points: the first lavage was after PaO_2 values decreased to <200 mmHg, followed by a second lavage 60 min after the first. The amount of saline used in all bronchoalveolar lavages was always 30 ml/kg body weight at 37°C; the amount of surfactant in the diluted surfactant suspension (Group V) was 3.3 mg phospholipids/ml (100 mg phospholipids/kg body weight). The amount of surfactant used for intratracheal instillation in Groups II and III was 200 mg phospholipids/kg body weight at a

concentration of 50 mg phospholipids/ml.

Blood samples for measurement of PaO_2 and $PaCO_2$ were taken from the carotid artery of each rat before intratracheal HCl (or saline) instillation, at regular intervals post-instillation and at 5, 30 and 60 min after treatment (ABL 330; Radiometer, Copenhagen, Denmark). In Groups IV and V blood gas analyses were also performed at 5, 30 and 60 min after the second lavage.

Bronchoalveolar lavage fluid

Bronchoalveolar lavage (BAL) was performed in the following groups for measurement of total protein concentration and surface tension properties. At the end of the experiments (i.e. 60 min after the second BAL) Groups IV and V were lavaged for the third time with saline (37 °C; 30 ml/kg). BAL fluids of Group III and the 1st, 2nd and 3rd BAL fluids from Groups IV and V were prepared as described below. To compare all BAL samples with BAL samples from healthy control animals, the rats of the control group were lavaged 2 h after saline instillation. The BAL fluids of the different groups were prepared as follows: all samples were centrifugated for 15 min at 2000 g to remove cell material. Protein concentration was measured in all samples using a modified Lowry method (22), with bovine serum albumin as standard. Also, surface activity in BAL fluid was measured with the Wilhelmy balance (E. Biegler GmbH, Mauerbach, Austria) by applying 500 µl of BAL fluid to the surface of a saline-filled trough. Surface area was reduced and expanded at a cycle speed of 0.33/min. Maximal and minimal surface tensions were measured after 3 cycles at 100% and 20% surface area, respectively.

Statistical analysis

All data are expressed as mean ± standard deviation (SD). For PaO_2 and $PaCO_2$ data, a two-factor mixed-design ANOVA (group by repeated measures [time]) was used. For analysis of BAL parameters, a one-way between-subjects ANOVA (group) was used. When significant differences between and/or within groups occurred, these differences were further analyzed (Student-Newman-Keuls test). Statistical significance was accepted at $p \leq 0.05$.

RESULTS

Animal study

Figure 1 shows PaO_2 values for each group. Before instillation of HCl, PaO_2 values are high (536.3 ± 29.9 mmHg) and there is no significant intergroup difference. HCl instillation decreased PaO_2 values to approximately 140 mmHg ("Pre"). After treatment there is a statistically significant increase in PaO_2 values in rats undergoing BAL followed by surfactant (Group III) and rats lavaged with the surfactant suspension (Group V). PaO_2 values did not increase in the other groups. The PaO_2 values of control rats receiving saline instead of HCl remained high after 2 h of ventilation (550.5 ± 27.7 mmHg). For other statistically significant differences, see Figure 1.

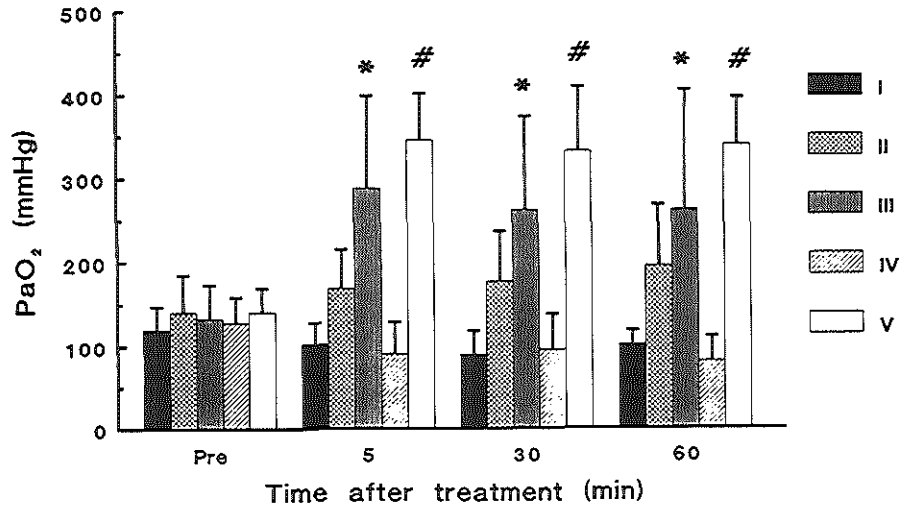


Figure 1. PaO_2 values (mean ± SD) of different groups after HCl instillation; after PaO_2 values decreased below 200 mmHg (=Pre) rats were divided into 5 groups: Group I received no treatment, Group II received surfactant (200 mg/kg), Group III was lavaged with saline followed by surfactant treatment (200 mg/kg); Groups IV and V were lavaged with saline or a diluted surfactant suspension (3.3 mg/ml), respectively; *=statistically significant differences between Group III and pre-treatment, Group I and Group IV; #=statistically significant differences between Group V and pre-treatment, Groups I, II and IV; PaO_2 = arterial oxygen tension.

Table 1 shows the $PaCO_2$ values for each group. There are no significant differences in $PaCO_2$ values between all groups at any time. $PaCO_2$ values remained low in control rats which received saline instead of HCl (36.5 ± 3.50 mmHg).

Table 2 shows the blood gases of rats lavaged with saline, and rats lavaged with the surfactant suspension (Groups IV and V, respectively) after the first and second lavage. After the second lavage there appears to be an increase in PaO_2 values in both groups compared to PaO_2 values after the first lavage, however, this increase is not significant. For statistically significant differences, see Table 2.

Table 1: $PaCO_2$ values (mmHg) of all groups.

Group	n	control	pre	5'	30'	60'
I	7	30.7 ± 3.19	58.1 ± 15.8	53.0†† ± 11.6	62.4 ± 23.1	76.0†† ± 13.6
II	8	34.8 ± 4.58	67.5 ± 20.3	67.5 ± 13.9	68.6 ± 10.3	65.6† ± 8.81
III	7	32.6 ± 4.78	65.1 ± 15.8	65.9 ± 23.4	57.3 ± 15.2	57.4 ± 18.7
IV (1^{st})	8	31.5 ± 3.61	57.6 ± 13.3	52.3 ± 8.10	49.9 ± 10.3	54.3†† ± 4.12
V (1^{st})	8	33.1 ± 3.25	65.1 ± 16.4	63.8 ± 10.8	57.3 ± 8.66	57.1† ± 5.32

All data are mean ± SD; Control = before HCl instillation; pre = after HCl instillation and before treatment; 5', 30' and 60' = min after treatment; IV (1^{st}) and V (1^{st}) = $PaCO_2$ values after the first BAL treatment; † = one animal died.

Table 2: PaO_2 and $PaCO_2$ values (mmHg) after repeated BAL.

Group		n	control	pre	1st BAL 5'	1st BAL 30'	1st BAL 60'	2nd BAL 5'	2nd BAL 30'	2nd BAL 60'
IV ($1^{st}/2^{nd}$)	PaO_2	8	539.1 ± 25.8	128.3 ± 30.9	90.3 ± 38.7	95.1 ± 43.1	81.0†† ± 30.7	99.8†† ± 32.5	131.4 ± 57.9	129.5 ± 68.1
	$PaCO_2$		31.5 ± 3.61	57.6 ± 13.3	52.3 ± 8.10	49.9 ± 10.3	54.3 ± 4.12	41.8* ± 10.9	42.1 ± 7.98	42.5 ± 9.21
V ($1^{st}/2^{nd}$)	PaO_2	8	536.0 ± 30.7	144.7 ± 28.8	345.2# ± 56.5	332.2 ± 77.4	340.0† ± 56.8	411.0 ± 45.3	418.9 ± 53.7	388.5† ± 87.6
	$PaCO_2$		33.1 ± 3.25	65.1 ± 16.4	63.8 ± 10.8	57.3 ± 8.66	57.1 ± 5.32	55.6 ± 7.26	50.9 ± 5.88	53.7 ± 6.93

All data are mean ± SD; IV and V ($1^{st}/2^{nd}$) = PaO_2 and $PaCO_2$ values after the first and second BAL treatment; Control = before HCl instillation; pre = after HCl instillation and before BAL treatment; 5', 30' and 60' = min after first and second BAL treatment; *$p<0.05$ between 1^{st} and 2^{nd} BAL; #$p<0.05$ between pre-BAL and 5 min after BAL; † = one animal died.

Bronchoalveolar lavage fluid

Table 3 gives the recovery percentage, protein concentration and surface tension properties of BAL fluid samples. The control lavages are from rats which received saline instead of HCl. The protein concentrations in BAL samples of control animals are much lower compared to all other groups, whereas there are no differences between the other groups. Maximal and minimal surface tensions of the BAL samples of rats lavaged with a diluted surfactant suspension are significantly lower compared to all other groups. The minimal surface tensions of BAL samples of sick animals lavaged with saline are significantly higher compared to healthy controls.

Table 3: BAL parameters of different groups.

Sample	n	Recovery [%]	Protein [mg/ml]	Surface tension [mN/m]	
				maximal	minimal
III	5	97.0 ± 2.45	7.14 ± 1.26	59.7# ± 1.82	32.1# ± 1.00
IV (1st)	5	100.0 ± 8.16	7.95 ± 1.29	60.1@ ± 0.41	32.2§ ± 0.42
V (1st)	6	97.5 ± 3.82	7.50 ± 0.97	54.9¶ ± 3.28	16.6¶ ± 2.19
Control	6	94.2 ± 5.32	0.40* ± 0.39	63.1 ± 3.16	24.9 ± 2.55

All data are mean ± SD; Control = BAL fluid of control rats receiving saline instead of HCl; IV (1st) and V (1st) = fluid from first BAL treatment; *p<0.01 between Control and other groups; #p<0.05 between maximal/minimal surface tension of III and other sample groups, except IV (1st); @p<0.05 between IV (1st) and V (1st); §p<0.05 between IV (1st) and other sample groups, except III; ¶p<0.05 between V (1st) and other groups.

Table 4 gives the protein concentrations in the 1st, 2nd and 3rd BAL samples from Groups IV and V. In both groups, there is a significant decrease in protein concentrations

between the 1st and the 3rd BAL samples. The difference in protein concentrations between Groups IV (3rd BAL) and V (3rd BAL) is statistically significant. There is no statistically significant difference in either maximal or minimal surface tensions between the 1st, 2nd and 3rd BAL samples of Group IV; the same is true for Group V (data not shown). The differences in surface tension between BAL samples of Groups IV and V, as shown in Table 3, also exist for the second and third BAL.

Table 4: Protein concentrations (mg/ml) in 1st, 2nd and 3rd BAL.

	IV	V
1st BAL	7.95 ± 1.29*	7.50 ± 0.97#
2nd BAL	6.11 ± 0.96	5.56 ± 0.84
3rd BAL	5.36 ± 0.70	3.99 ± 0.75@

All data are mean ± SD; *$p<0.05$ between 1st BAL and 3rd BAL; #$p<0.05$ between 1st BAL and both 2nd and 3rd BAL; @$p<0.05$ between IV (3rd) and V (3rd).

DISCUSSION

Massive aspiration of HCl with low pH leads to damage of the alveolar-capillary membrane which in turn leads to increased permeability to, and intra-alveolar accumulation of, plasma proteins (3-6). These proteins have proven to be potent surfactant inhibitors (7-13). The resulting impaired surfactant function leads to accumulation of edema fluid in the alveolar space. Also, as a result of diminished surfactant function, surface tension on the alveolar wall increases, leading to increased retractive forces across the alveolar-capillary membrane, with subsequent formation of atelectasis and mismatch of the ventilation-perfusion ratio, leading to hypoxemia. The damage to the surfactant system and the fact that surfactant is no longer produced as a result of damage to the surfactant producing type II pneumocytes (4), makes treatment of this pathological entity with surfactant plausible.

The results in the present study demonstrate that ventilation with 100% oxygen and increased inflation pressure and PEEP to the levels used in this study, were not able

to maintain high PaO_2 levels in rats after HCl aspiration. Also, surfactant instillation without prior lavage did not restore lung function. The best way to treat these animals appears to be either to lavage the lungs with saline, directly followed by intratracheal instillation of a high dose of surfactant, or to lavage the lungs with a diluted surfactant suspension, as evidenced by an increase in PaO_2.

The decrease in PaO_2 values after HCl aspiration could not be explained by a fluid challenge, as demonstrated by PaO_2 values remaining high in rats receiving saline instead of HCl.

It has been hypothesized that the mechanism of inhibition of surfactant by proteins is based on competition for space at the air-liquid interphase (9,12). Thus, the way to overcome the inhibition of surfactant by these proteins would be to get a relatively high surfactant concentration in relation to the amount of proteins. This favorable surfactant/inhibitor (S/I) ratio can be achieved in two ways. In the first place by treating the animals with a large dose of surfactant (without prior lavage), a high S/I ratio could be achieved. The results from the present study demonstrate no increase in PaO_2 values after instillation of surfactant at a dose of 200 mg/kg in animals suffering from respiratory failure after HCl aspiration, although a further decrease in PaO_2 values seemed to be prevented at this surfactant concentration. Also, in comparable studies on animals suffering from respiratory failure due to HCl aspiration, surfactant only was not able to restore gas exchange (6,15). A possible explanation is that surfactant, at the concentration used, is directly inhibited by high concentration of inhibitors. Another explanation could be that the edema fluid and the atelectatic areas form a mechanical barrier, preventing surfactant from entering the alveolar spaces. However, in another study, surfactant instillation at very high doses (280-350 mg/kg) was able to restore gas exchange in guinea pigs suffering from severe respiratory failure due to protein-rich lung edema after intravenous instillation of anti-lung serum (23). Thus, in the latter study a more favorable S/I ratio was obtained by giving a large amount of surfactant. Recently, Kobayashi and colleagues (24) investigated the ability of surfactant mixed with edema fluid at several ratios to restore lung function in immature rabbit fetuses, as measured by tidal volume at preset insufflation pressures. It was demonstrated that surfactant (25 mg/ml) mixed with edema fluid at a protein to lipid ratio (P/L ratio) of 2.2 was capable of restoring lung function, whereas surfactant mixed with edema fluid at P/L ratio of

11.2, was not. Surface tension properties of these mixtures demonstrated high minimal surface tensions at P/L≥3.4 and low surface tensions at P/L≤1.8.

In the second place a favorable S/I ratio could be achieved by removing the inhibitors from the alveolar space by means of BAL. In the present study PaO_2 values increased after surfactant treatment (200 mg/kg) following lung lavage. Similar findings were also reported by Kobayashi and colleagues (6). In our study, although there was no statistically significant difference in PaO_2 values with rats receiving surfactant after BAL, BAL with a diluted surfactant suspension (100 mg/kg) seemed to be more efficient in restoring gas exchange. BAL with saline alone (without any additional surfactant treatment) was not able to restore gas exchange. The difference between PaO_2 values of rats lavaged with a diluted surfactant suspension and rats lavaged with saline (not followed by surfactant treatment) may be explained as follows: after removal of a large amount of proteins in both groups, a small amount of surfactant remains in the lungs of the rats lavaged with a diluted surfactant suspension. This appears sufficient to establish surface active material at the air-liquid interphase, allowing improved gas exchange across the alveolar-capillary membrane. As no surfactant concentration measurements were made in the recovered surfactant suspensions after BAL, no exact data is available on the amount of surfactant remaining in the lungs after BAL. If one tries to estimate the amount of surfactant remaining in the lungs, the following equations can be made: the functional residual capacity (FRC) of the lungs is approximately 10 ml/kg; BAL was performed with 30 ml/kg and almost 100% of the BAL fluid was recovered; the total amount of surfactant in the BAL fluid was 100 mg phospholipids/kg; this was diluted with 10 ml/kg of residual (edema) fluid in the lungs. Considering the mixture of these two concentrations (10 ml/kg= no surfactant; 30 ml/kg= 100 mg surfactant), this means that approximately 25 mg phospholipids/kg remains in the lungs. Considering the amount of surfactant needed to treat respiratory failure due to HCl aspiration, half the amount of surfactant is needed when BAL is performed with the diluted surfactant suspension, compared to treatment with surfactant after BAL with saline.

Protein concentrations in BAL material from all rats suffering from respiratory failure due to HCl aspiration were significantly higher compared to protein concentrations in BAL material from healthy control animals. Also, surface tension measurements demonstrated high minimal surface tensions in BAL material from rats

suffering from respiratory failure after HCl aspiration, when compared to BAL material from healthy controls. These results indicate that the surfactant system is damaged in animals after HCl aspiration. In BAL material from rats which underwent multiple lavage, the protein concentration decreased significantly, both in rats lavaged with the diluted surfactant suspension and in those lavaged with saline (Table 4). In rats lavaged with the diluted surfactant suspension, the decrease in protein concentration was greater compared to those lavaged with saline. Perhaps this indicates repair of the alveolar-capillary membrane, resulting in a decrease of protein influx into the alveoli. PaO_2 values appeared to increase further after the second lavage with the diluted surfactant suspension, although this increase was not statistically significant (Table 2). Thus, although BAL with saline also removes the proteins from the alveolar spaces, surfactant substitution is needed to restore lung function. These results suggest that surfactant inhibitors (e.g. plasma-derived proteins, specific proteases and cellular breakdown products) can be removed by means of BAL, resulting in improved efficacy of surfactant in restoring lung function.

In conclusion: intratracheal surfactant instillation without prior BAL does not improve pulmonary gas exchange in rats suffering from respiratory failure due to HCl aspiration; in these animals gas exchange could be improved after BAL with saline, followed directly by surfactant instillation, or by BAL with a diluted surfactant suspension. It is shown that (multiple) BAL removes edema fluid (containing plasma proteins) from the lungs. It is argued that surfactant treatment can only succeed when there is a favorable ratio of surfactant to proteins inhibiting surfactant function.

It is difficult to extrapolate from this animal study to the clinical situation. However, if one decides to treat patients suffering from ARDS with surfactant, it should be considered that the amount of surfactant needed to overcome the inhibition by proteins is enormous. From this it could be speculated that it would probably be more effective to reduce the amount of intra-alveolar proteins by means of BAL and then treat with surfactant, or directly by means of BAL with a diluted surfactant suspension. However, the optimal way to perform this in patients has yet to be studied.

REFERENCES

1. Gibbs CP, Modell JH: Management of aspiration pneumonitis, Anesthesia. Edited by Miller RD. New York, Churchill Livingstone, 1990, pp 1293-1319
2. Fowler AA, Hamman RF, Good JT, Benson KN, Baird M, Eberle DJ, Petty TL, Hyers TM: Adult respiratory distress syndrome: risk with common predispositions. Ann Intern Med 98: 593-597, 1983
3. Awe WC, Fletcher WS, Jacob SW: The pathophysiology of aspiration pneumonitis. Surgery 60: 232-239, 1966
4. Greenfield LJ, Singleton RP, McCaffree DR, Coalson JJ: Pulmonary effects of experimental graded aspiration of hydrochloric acid. Ann Surg 170: 74-86, 1969
5. Grimbert FA, Parker JC, Taylor AE: Increased pulmonary vascular permeability following acid aspiration. J Appl Physiol 51: 335-345, 1981
6. Kobayashi T, Ganzuka M, Tanigushi J, Nitta K, Murakami S: Lung lavage and surfactant replacement for hydrochloric acid aspiration in rabbits. Acta Anaesthesiol Scand 34: 216-221, 1990
7. Ennema JJ, Kobayashi T, Robertson B, Curstedt T: Inactivation of exogenous surfactant in experimental respiratory failure induced by hyperoxia. Acta Anaesthesiol Scand 32: 665-671, 1988
8. Fuchimukai T, Fujiwara T, Takahashi A, Enhorning G: Artificial pulmonary surfactant inhibited by proteins. J Appl Physiol 62: 429-437, 1987
9. Holm BA, Enhorning G, Notter RH: A biophysical mechanism by which plasma proteins inhibit lung surfactant activity. Chem Phys Lipids 49: 49-55, 1988
10. Ikegami M, Jobe A, Jacobs H, Lam R: A protein from airways of premature lambs that inhibits surfactant function. J Appl Physiol 57: 1134-1142, 1984
11. Seeger W, Stöhr G, Wolf HRD, Neuhof H: Alteration of surfactant function due to protein leakage: special interaction with fibrin monomer. J Appl Physiol 58: 326-338, 1985
12. Holm BA, Notter RH: Effects of hemoglobin and cell membrane lipids on pulmonary surfactant activity. J Appl Physiol 63: 1434-1442, 1987
13. Winn R, Stothert J, Nadir B, Hildebrandt J: Lung Mechanics following aspiration of 0.1 N hydrochloric acid. J Appl Physiol 55: 1051-1056, 1983
14. Lamm WJE, Albert RK: Surfactant replacement improves lung recoil in rabbit lungs after acid aspiration. Am Rev Respir Dis 142: 1279-1283, 1990
15. Strohmaier W, Redl H, Schlag G: Studies of the potential role of a semisynthetic surfactant preparation in an experimental aspiration trauma in rats. Exp Lung Res 16: 101-110, 1990
16. Eijking EP, Gommers D, So KL, De Maat MPM, Mouton JW, Lachmann B: Prevention of respiratory failure after hydrochloric acid aspiration by intratracheal surfactant instillation in rats. Anesth Analg 76: 472-477, 1993
17. Van Daal GJ, So KL, Gommers D, Eijking EP, Fiévez RB, Sprenger MJ, Van Dam DW, Lachmann B: Intratracheal surfactant administration restores gas exchange in experimental adult

respiratory distress syndrome associated with viral pneumonia. Anesth Analg 72: 589-595, 1991

18. Eijking EP, Van Daal GJ, Tenbrinck R, Luijendijk A, Sluiters JF, Hannappel E, Lachmann B: Effect of surfactant replacement on pneumocystis carinii pneumonia in rats. Intensive Care Med 17: 475-478, 1991

19. Van Daal GJ, Bos JAH, Eijking EP, Gommers D, Hannappel E, Lachmann B: Surfactant replacement therapy improves pulmonary mechanics in end-stage influenza A pneumonia in mice. Am Rev Respir Dis 145: 859-863, 1992

20. Bos AP, Tibboel D, Hazebroek FWJ, Molenaar JC, Lachmann B, Gommers D: Surfactant replacement therapy in high-risk congenital diaphragmatic hernia (Letter). Lancet (1) 338: 1279, 1991

21. Metcalfe IL, Enhorning G, Possmayer F: Pulmonary surfactant-associated proteins: their role in the expression of surface activity. J Appl Physiol 49: 34-41, 1980

22. Markwell MAK, Haas SM, Bieber LL, Tolbert NE: A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal Biochem 87: 206-210, 1978

23. Lachmann B, Hallman M, Bergmann KC: Respiratory failure following anti-lung serum: study on mechanisms associated with surfactant system damage. Exp Lung Res 12: 163-180, 1987

24. Kobayashi T, Nitta K, Ganzuka M, Inui S, Grossmann G, Robertson B: Inactivation of exogenous surfactant by pulmonary edema fluid. Pediatr Res 29: 353-356, 1991

CHAPTER 7

EFFECT OF SURFACTANT REPLACEMENT ON PNEUMOCYSTIS CARINII PNEUMONIA IN RATS

E.P. Eijking, G.J. van Daal, R. Tenbrinck, A. Luijendijk*, J.F. Sluiters*, E. Hannappel# and B. Lachmann

Depts. of Anesthesiology and *Clinical Microbiology, Erasmus University, Rotterdam, The Netherlands, #Institute of Biochemistry, University of Erlangen, Erlangen, FRG

In: Intensive Care Med 1991; 17: 475-478

This topic was presented at the 5th European Congress on Intensive Care Medicine in Amsterdam in June 1990. An award was received for this presentation.

SUMMARY

The effect of intratracheal surfactant instillation on pulmonary function in rats with pneumocystis carinii pneumonia (PCP) was investigated. In those animals which developed PCP with severe respiratory failure after s.c. administration of cortisone acetate over 8-12 weeks, pulmonary function was improved by surfactant instillation. PaO_2 values 30 min after surfactant instillation were significantly higher compared to pre-treatment values and also compared to PaO_2 values of rats 30 min after receiving saline (482.9 mmHg ± 44.7, 170.7 mmHg ± 39.3 and 67.2 mmHg ± 17.4, respectively). Histological examination showed that alveoli of rats with PCP which received no exogenous surfactant are filled with foamy edema, whereas after exogenous surfactant alveoli are stabilized and well-aerated. These results indicate that exogenous surfactant may help patients with severe PCP to overcome an acute stage of respiratory distress.

INTRODUCTION

Infectious diseases are of great concern in intensive care health service in immunocompromized patients; especially infectious diseases of the lung. In these patients pneumocystis carinii is a major cause of pneumonia leading to severe respiratory distress [1,2]. It has been demonstrated in rats that pneumocystis carinii pneumonia (PCP) decreases the total amount of phospholipids in bronchoalveolar lavage (BAL) fluid whereas phospholipase activity in BAL fluid increases [3,4]. Furthermore, lung compliance also decreases in PCP [3]. These findings suggest that in the pathophysiology of respiratory failure in PCP the pulmonary surfactant system may be involved.

It has been repeatedly demonstrated by several authors that intratracheal instillation of exogenous surfactant restores lung function in animals with respiratory failure due to surfactant depletion or intratracheal HCl administration [5,6]. Also large numbers of neonates with respiratory distress syndrome (RDS) due to lack of surfactant have been succesfully treated with exogenous surfactant [7,8,9,10].

We designed a study to investigate the effect of surfactant replacement therapy on pulmonary function in rats with PCP. If surfactant replacement would increase gas exchange, then one could conclude that a disturbed surfactant system is one of the

factors in the pathophysiology of respiratory failure in PCP.

MATERIALS AND METHODS

Surfactant

The surfactant used in these experiments is a natural surfactant isolated from bovine lungs in basically the same manner as previously described [11]. It consists of approximately 83% phospholipids, 1% hydrophobic proteins (SP-B and SP-C), the remainder being other lipids such as cholesterol, glyceride and free fatty acids. There is no SP-A in this surfactant preparation.

Animal model

This protocol was approved by the Animal Care and Use Committee of the Erasmus University Rotterdam, The Netherlands.

The studies were performed in 28 male Wistar rats (initial body weight: 110-120 g), placed in two groups. The first group (n=20) was treated with cortisone acetate, based on the model described by Frenkel and Chandler [12,13]. These animals received 12.5 mg cortisone acetate s.c. four times weekly over 8-12 weeks and 10 μg/ml doxycycline in drinking water to reduce a possible bacterial superinfection. The second group (n=8) received no treatment and served as controls. All animals were housed in standard plastic cages and received standard food *ad libitum* and were weighed at weekly intervals.

After 8-12 weeks the cortisone acetate treated animals developed respiratory failure as judged by clinical symptoms (e.g. tachypnoea, cyanosis and fast decrease in body weight). Eleven animals died during the night hours on various days before treatment with exogenous surfactant could be started. Nine rats which suffered clinically from respiratory failure were anesthetized with pentobarbital sodium (60 mg/kg, i.p.), tracheotomized and paralyzed with pancuronium bromide (0.5 mg/kg, i.m.). A catheter was inserted into the carotid artery for blood sampling. Animals were ventilated pressure-controlled with a Servo Ventilator 900 C (Siemens-Elema, Solna, Sweden) at the following settings: FiO_2 = 1.0, ventilation frequency = 30/min, peak airway pressure = 22 cm H_2O, PEEP = 2 cm H_2O and I:E = 1:2.

After a ± 20 min stabilization period, 6 animals received bovine surfactant (200 mg dry weight/kg body weight), suspended in 0.7 ml saline, intratracheally, whereas 3 received the same amount of saline. Blood samples were taken from the carotid artery immediately before surfactant instillation and at 30 and 60 min post treatment. Blood gases were measured with the ABL 330 Acid-Base Laboratory (Radiometer, Copenhagen, Denmark).

Histological examination

At the end of the experiments the animals were sacrificed with an overdose of intra-arterially administered pentobarbital. The lungs were removed, the left lobe was minced and a cytospin preparation was made. This preparation was stained with a modified Gomori stain [14], which allows visualization of the cysts (not the intra-cystic structures) and Giemsa stain, and examined to confirm presence of PCP. The right lobes were fixed in 10% formalin for light microscopy examination, dehydrated, embedded in paraffin and 6 μm sections of the right middle lobe were stained with hematoxylin and eosin, Grocott-Gomori methenamine silver nitrate and periodic acid-Schiff stain.

Statistical analysis

Statistical evaluation of data was performed using the Wilcoxon signed rank test for paired observations or the Mann-Whitney-U test (two-tailed). All data are expressed as mean ± SEM. Statistical significance was accepted at $p < 0.05$ (two-tailed).

RESULTS

Blood gases

During an 8-12 week period the animals developed respiratory insufficiency which could be reversed by surfactant instillation. PaO_2 values at 30 and 60 min after surfactant instillation are significantly higher compared to pre-treatment values. There is no significant difference between PaO_2 values after surfactant treatment and PaO_2 values of healthy control animals, though the latter are somewhat higher. Intratracheal instillation of only saline, however, led to a decrease of PaO_2 compared to pre-treatment PaO_2 values. The difference at 30 and 60 min between rats receiving surfactant and rats

receiving saline is significant. Pre-treatment PaO_2 values of both groups do not differ significantly (Fig. 1). Figure 2 shows one example of a dramatic increase of PaO_2 of a rat with PCP treated with exogenous surfactant. This rat had the lowest PaO_2 of the whole group.

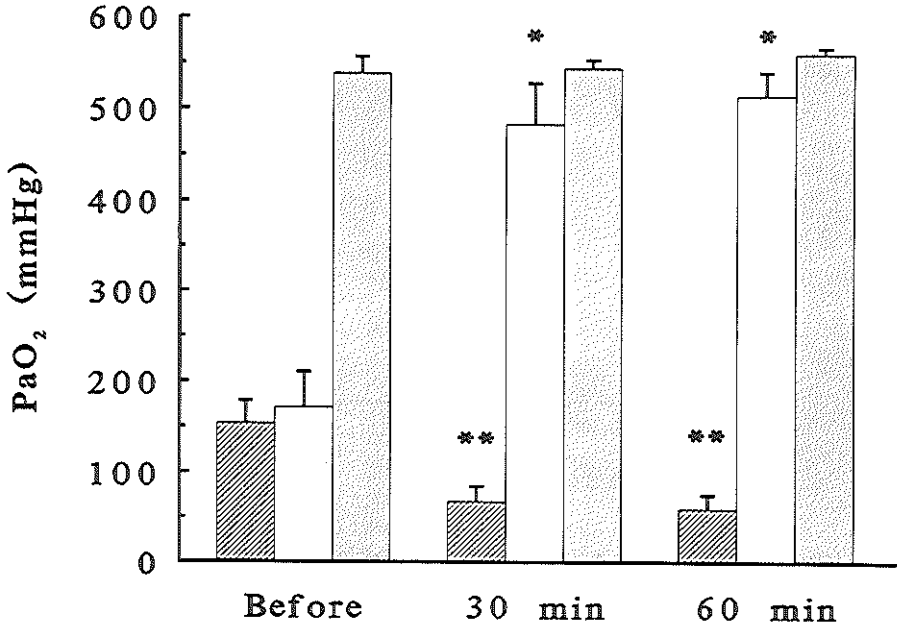


Figure 1. PaO_2 values of the different groups before treatment ("Before") and 30 min and 60 min after treatment (respectively: "30 min" and "60 min"). Grey bar = healthy controls; striped bar = saline group; open bar = exogenous surfactant group. (* = significantly different from pre-treatment; ** = significantly different from surfactant treated rats [$p<0.05$]).

Histologic findings

Histological examination of lungs of rats with PCP which received only saline and no exogenous surfactant showed alveoli filled with a characteristic foamy edema (Fig. 3). Surfactant treatment resulted in stabilized and aerated alveoli, thus allowing the emphysematous structure of the lungs to become apparent (Fig. 4).

In all 9 lung homogenates a large amount of cysts was present which confirmed the presence of PCP in all animals.

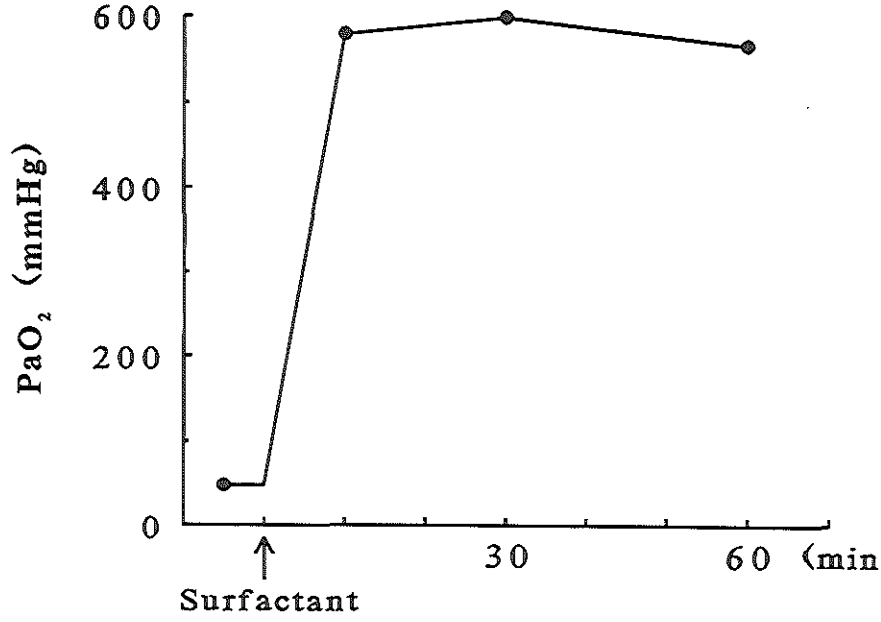


Figure 2. PaO_2 over time in a rat with PCP having the lowest PaO_2 of the whole group before surfactant treatment: in this animal PaO_2 was also measured 10 min after surfactant instillation. The arrow indicates the time of surfactant instillation.

DISCUSSION

The treatment of choice in patients suffering from respiratory failure due to PCP is administration of antimicrobial drugs combined with ventilatory support. The aim of the present study was to investigate the effect of an exogenous surfactant preparation on gas exchange under unchanged ventilator settings in an animal model of PCP.

The primary functions of surfactant are stabilization of alveoli of different size, and reduction of work of breathing by reducing surface tension at the air-liquid interphase on the alveolar walls [15]. In this study two hypotheses were proven valid:

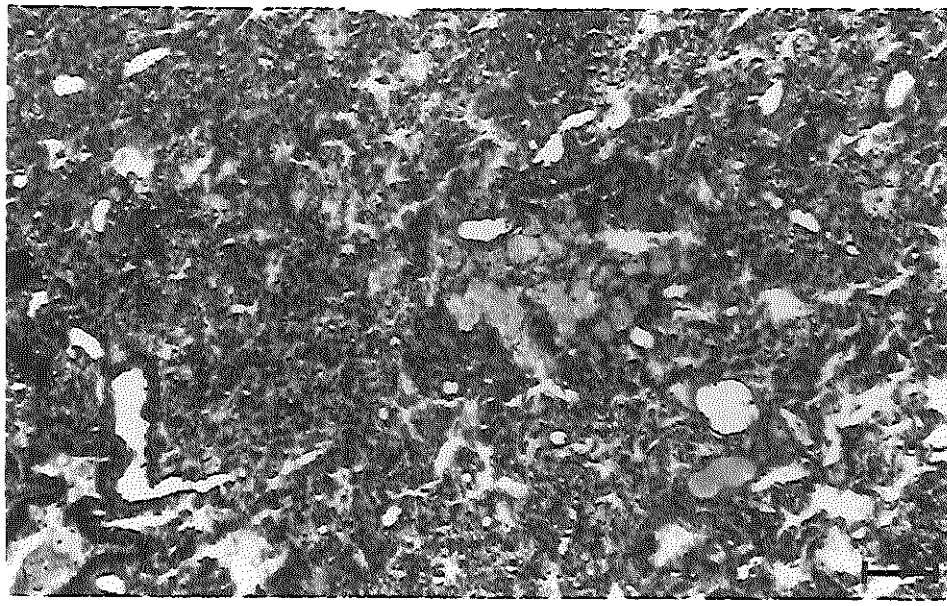


Figure 3. Typical histological findings in a rat with PCP which received saline instead of exogenous surfactant. Hematoxylin and eosin stain; the scale-line equals 100 μm.

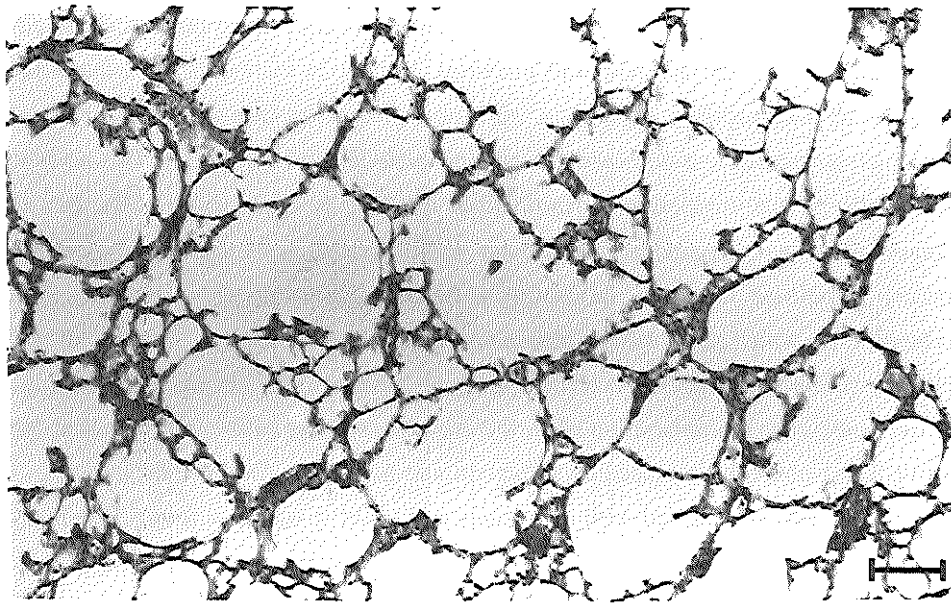


Figure 4. Typical histological findings in rats with PCP after treatment with surfactant; note the emphysematous structure. Hematoxylin and eosin stain; the scale-line equals 100 μm.

hypothesis 1: if surfactant is necessary to keep retractive forces as low as possible and to ensure optimal gas exchange, any disturbance in the surfactant system will result in abnormalities in lung distensibility and gas exchange; *hypothesis 2:* if the first hypothesis is correct, surfactant replacement will restore lung distensibility and gas exchange to normal. In other words, if surfactant replacement improves decreased lung compliance and impaired gas exchange in lung diseases of different etiologies, then the surfactant system is also involved in the pathogenesis of these lung diseases [16].

The results of this study demonstrate that surfactant instillation in rats with respiratory failure due to pneumoncystis carinii infection leads to restoration of pulmonary gas exchange, which could be demonstrated by improvement of blood gases and by histological examination. These results underline our hypothesis that the pulmonary surfactant system is at least partly responsible for the respiratory failure caused by PCP. The mechanism can be explained as follows: as a result of damage to the alveolar-capillary membrane caused by infection with pneumocystis carinii, large volumes of edema fluid accumulate into the alveolar space. This edema contains large amounts of plasma proteins and specific enzymes capable of disrupting the surfactant system [3,4,17,18,19]. As a result of this functional surfactant deficit, surface tension at the air-liquid interphase on the alveolar wall increases, leading to increased retractive force across the alveolar-capillary membrane, with subsequent formation of atelectatic areas, functionally expressed as a decrease in functional residual capacity (FRC). This leads to a mismatch of the ventilation-perfusion ratio resulting in hypoxaemia. Also, due to increase of surface tension on the alveolar walls, suction force across the alveolar-capillary membrane increases, facilitating a further outpouring of edema [20]. The mechanism by which surfactant replacement therapy leads to improvement of gas exchange can be explained by reversing these mechanisms. The fact that gas exchange improves only after administration of surfactant and not after saline indicates that the positive effect observed is caused by surfactant and not by the fluid itself.

In all animals treated with exogenous surfactant it was also found that many alveolar septa were damaged, leading to an emphysematous lung structure. Similar observations were reported recently in patients with PCP [21]. These findings suggest that in PCP lungs proteolytic enzymes may be over-represented. These proteolytic enzymes are perhaps excreted by activated leukocytes attracted to the site of inflamation. Also in

the past it has been found that type I pneumocytes undergo degenerative changes in rats infected with pneumocystis carinii. This leads to damage to the ultrastructure of the alveolar wall, thus leading to increased permeability of the alveolar-capillary membrane [22]. Perhaps both mechanisms play a role in the damage of the alveolar septa. It could be speculated that proteinase inhibitors, given to the patient after manifestation of pneumocystis carinii infection, could prevent (further) morphological damage to the pulmonary structure.

It is established that immunosuppressed rats, as is the case in humans, can develop pneumocystis carinii infection of the lungs, leading to respiratory distress. The current treatment of choice is parenteral administration of trimethoprim-sulfamethoxazole or pentamidine-isothionate. Both drugs have about the same rate of success of around 75% (percentages vary depending on the study) but high incidences of systemic adverse reactions are reported (for review see: [23]). To prevent these adverse reactions it is necessary to lower the systemic concentration, which led to studies on aerosolized pentamidine. Results from these aerosol studies have shown that it is possible to get high concentrations in the lungs with low systemic concentrations [24,25]. For the future one could speculate to use surfactant as a carrier-substance for antimicrobial drugs, this way achieving high intra-alveolar drug concentration and low systemic concentration.

In conclusion, it has been demonstrated that surfactant instillation can improve gas exchange in rats suffering from respiratory failure due to PCP. These results could be important in future for treatment regimens of patients suffering from acute respiratory failure due to PCP.

REFERENCES

1. CDC Update (1984) acquired immuno-deficiency syndrome (AIDS)-United States. MMWR 32: 688-691
2. Walzer PD, Perl DP, Krogstad DJ, Rawson PG, Schultz MG (1974) Pneumocystis carinii pneumonia in the United States: epidemiologic, diagnostic, and clinical features. Ann Intern Med 80: 83-93
3. Sheehan PM, Stokes DC, Yeh Y, Hughes WT (1986) Surfactant phospholipids and lavage phospholipase A_2 in experimental pneumocystis carinii pneumonia. Am Rev Respir Dis 134: 526-

531

4. Kernbaum S, Masliah J, Alcindor LG, Bouton C, Christol D (1983) Phospholipase activities of bronchoalveolar lavage fluid in rat pneumocystis carinii pneumonia. Br J Exp Path 64: 75-80
5. Berggren P, Lachmann B, Curstedt T, Grossmann G, Robertson B (1986) Gas exchange and lung morphology after surfactant replacement in experimental adult respiratory distress syndrome induced by repeated lung lavage. Acta Anaesth Scand 30: 321-328
6. Kobayashi T, Ganzuka M, Taniguchi J, Nitta K, Murakami S (1990) Lung lavage and surfactant replacement for hydrochloric acid aspiration in rabbits. Acta Anaesthesiol Scand 34: 216-221
7. Fujiwara T, Chida S, Watabe Y, Maeta H, Morita T, Abe T (1980) Artificial surfactant therapy in Hyaline-Membrane Disease. Lancet 1: 55-59
8. Hallman M, Merritt TA, Jarvenpaa A-L, Boynton B, Mannino F, Gluck L, Moore T, Edwards D (1985) Exogenous human surfactant for treatment of severe respiratory distress syndrome: a randomized clinical trial. J Pediatrics 106: 963-969
9. Collaborative European Multicenter Study Group (1988) Surfactant replacement therapy for severe neonatal respiratory distress syndrome: an international randomized clinical trial. Pediatrics 82: 683-691
10. Ten Centre Study Group (1987) Ten centre trial of artificial surfactant (artificial lung expanding compound) in very premature babies. Br Med J 294: 991-996
11. Metcalfe IL, Enhorning G, Possmayer F (1980) Pulmonary surfactant-associated proteins: their role in the expression of surface activity. J Appl Physiol 49: 34-41
12. Frenkel JK, Good JT, Schultz JA (1966) Latent pneumocystis infection of rats, relapse, and chemotherapy. Lab Invest 15: 1559-1577
13. Chandler FW, Frenkel JK, Campbell WG (1979) Animal model of human disease: pneumocystis pneumonia. Am J Path 95: 571-574
14. Musto L, Flanigan M, Elbadawi A (1982) Ten-minute silver stain for pneumocystis carinii and fungi in tissue sections. Arch Pathol Lab Med 106: 292-294
15. Clements JA (1977) Functions of the alveolar lining. Am Rev Respir Dis 115: 67-71
16. Lachmann B (1988) Surfactant replacement in acute respiratory failure: Animal studies and first clinical trials. In: Lachmann B (ed) Surfactant replacement therapy in neonatal and adult respiratory distress syndrome. Springer-Verlag, Berlin, pp 212-223
17. Ikegami M, Jobe A, Jacobs H, Lam R (1984) A protein from airways of premature lambs that inhibits surfactant function. J Appl Physiol 57: 1134-1142
18. Holm BA, Enhorning G, Notter RH (1988) A biophysical mechanism by which plasma proteins inhibit lung sufactant activity. Chem Phys Lipids 49: 49-55
19. Seeger W, Stöhr G, Wolf HRD, Neuhof H (1985) Alteration of surfactant function due to protein leakage: special interaction with fibrin monomer. J Appl Physiol 58: 326-338
20. Guyton AC, Moffatt DS, Adair TH (1984) Role of alveolar surface tension in transepithelial

movement of fluid. In: Robertson B, Van Golde LMG, Batenburg JJ (eds) Pulmonary surfactant. Elsevier Science Publishers, Amsterdam, pp 171-185

21. Beers MF, Sohn M, Swartz M (1990) Histological evidence of peripheral tissue destruction in AIDS patients with pneumocystis carinii pneumonia complicated by recurrent spontanuous pneumothorax. Am Rev Respir Dis 141 (Suppl): A273

22. Yoneda K, Walzer PD (1981) Mechanism of pulmonary alveolar injury in experimental pneumocystis carinii pneumonia in the rat. Br J Exp Path 62: 339-346

23. Levine SJ, White DA (1988) Pneumocystis carinii. Clin Chest Med 9 (3): 395-423

24. Montgomery AB, Debs RJ, Luce JM, Corkery KJ, Turner J, Brunette EN, Lin ET, Hopewell PC (1987) Aerosolized pentamidine as sole therapy for pneumocystis carinii pneumonia in patients with acquired immunodeficiency syndrome. Lancet 2: 480-483

25. Debs RJ, Blumenfeld W, Brunette EN, Straubinger RM, Montgomery AB, Lin E, Agabian N, Papahadjopoulos D (1987) Successful treatment with aerosolized pentamidine of pneumocystis carinii pneumonia in rats. Antimicrob Agents Chemother 31: 37-41

CHAPTER 8

IN VIVO AND IN VITRO INACTIVATION OF BOVINE SURFACTANT BY AN ANTI-SURFACTANT MONOCLONAL ANTIBODY

E.P. Eijking, D.S. Strayer*, G.J. van Daal, R. Tenbrinck, T.A. Merritt#, E. Hannappel@ and B. Lachmann

Depts. of Anesthesiology, Erasmus University, Rotterdam, The Netherlands; *Pathology and Laboratory Medicine, University of Texas Health Science Center, Houston, USA; #Pediatrics, University of California, San Diego, USA; @Institute for Biochemistry, University of Erlangen, Erlangen, FRG.

In: Eur Respir J 1991; 4: 1245-1250

SUMMARY

In this study the importance of a low-weight surfactant protein (11 kDa) is demonstrated by selectively blocking this protein with a monoclonal antibody. In adult rats respiratory failure was induced by repeated broncho-alveolar lavage to remove all pulmonary surfactant. It was shown that surfactant mixed with the antibody was not capable of restoring lung function when compared with surfactant alone or surfactant mixed with control serum. Using the pulsating bubble surfactometer, it could be demonstrated that surfactant mixed with this antibody had a significantly higher minimum surface tension when compared with surfactant alone, or surfactant mixed with an unrelated mouse-IgG. The inhibition of surfactant function by the monoclonal antibody suggests the importance of the 11 kDa protein for normal surfactant function.

INTRODUCTION

Pulmonary surfactant is mainly composed of specific phospholipids and proteins. Surfactant proteins have been divided into three groups: SP-A, SP-B and SP-C (for review, see [1,2]). Recently the presence of a collagenous protein, SP-D (MW 43 kDa, reduced), has been demonstrated in primary type II cell cultures. This protein has been proven to be immunologically and structurally different from SP-A and the low molecular weight surfactant proteins. The function of SP-D is still unknown [3].

SP-B and SP-C are low-weight hydrophobic proteins with overlapping molecular weights between 5 kDa and 18 kDa, depending on the animal species, the method of preparation and the state of reduction (for review, see [2]). Mixtures of phospholipids and low-weight hydrophobic proteins reduce surface tension and increase the effectiveness of exogenously administered phospholipids in fetal rabbits, as measured by an increased lung compliance [4,5,6]. On the other hand it has been demonstrated that monoclonal and polyclonal antibodies directed against surfactant can inhibit the function of surfactant in vitro, i.e. lowering surface tension and facilitating adsorption [7,8,9,10].

The role of hydrophobic surfactant proteins in surfactant function in adult animals remains unclear. In the present study a monoclonal anti-surfactant antibody was raised in rats. This monoclonal antibody recognizes an 11 kDa surfactant protein. The function

of this 11 kDa surfactant protein was investigated in vivo and in vitro, by selectively blocking the its function with the monoclonal antibody (C1).

MATERIALS AND METHODS

Surfactant

The surfactant used in these experiments is a natural surfactant isolated from bovine lungs in basically the same manner as previously described [11]. It consists of approximately 83% phospholipids, 1% hydrophobic proteins (SP-B and SP-C), the remainder being other lipids such as cholesterol, glyceride and free fatty acids. There is no SP-A in this surfactant preparation.

Monoclonal antibody

Monoclonal antibodies against bovine surfactant were raised in rats. Procedures used to raise, absorb and assay rat polyclonal antisera have been described in detail [9,12,13,14,15]. In brief: F344 rats received bovine surfactant emulsified in Freund's complete adjuvant (Difco) subcutaneously, followed by intravenous boosts. Antibody reactivity to surfactant was measured by ELISA [16] using antibody to mouse-Ig, conjugated to alkaline phosphatase (Cappel), and an automated ELISA reader (Dynatech). The animals showing high serum titers of antibody to bovine surfactant were boosted and their spleen cells fused to the nonsecreting rat myeloma line YB2/0 (ATCC). After growth in HAT (hypoxanthine + aminopterin + thymidine) containing medium for two weeks, positive wells were selected, subcloned and expanded. Resulting hybridomas were grown in RPMI-1640 with 10% normal horse serum (Hyclone). These hybridomas have been stable for over 100 passages in vitro. One of these hybridomas, C1, secretes the monoclonal antibody used in the present study. Using isotype-specific antiserum, this antibody was identified as IgG.

Western blotting

Western blot analysis of the specific protein reactivity of C1 was performed under reducing conditions according to procedures described elsewhere [17,18], with several modifications. Bovine surfactant proteins were electrophoresed in parallel lanes using

SDS-PAGE. All gels were 15% acrylamide, 0.1% SDS and 4 M urea. We transferred surfactant containing slab gel proteins to Immobilon® (Millipore Corp.), prepared according to package insert. All transfers were accomplished in standard transfer buffer [18], supplemented with 0.1% SDS. Filters were then cut into strips ("Spaghetti Westerns") and exposed to the antibody-containing culture supernatant (diluted 1:10), washed, and then exposed to rabbit anti-mouse IgG (1:20) (Miles Immunochemicals). Finally antibody-treated filter strips were incubated with 10^5 cpm of ^{125}I-Staphylococcus protein A per strip and visualized by autoradiography. Protein sizing was accomplished by interpolating between the positions of known molecular weight markers run in adjacent lanes. These procedures revealed that C1 recognizes an 11 kDa protein (Fig. 1).

Measurement of surface activity

The effect of the antibody on surface activity was studied in vitro using the pulsating bubble surfactometer, as described by Enhorning [19].

In brief: the surfactometer used in these in vitro experiments measures the pressure gradient across the air-liquid interphase of a bubble in a 37 °C water-enclosed sample chamber. The bubble size is viewed through a microscopic optic. The bubble pulsates between a maximal (0.55 mm) and a minimal (0.45 mm) radius at a rate of 20 cycles/min. The surface tension is calculated according to the law of Young and Laplace and expressed as milli Newton/meter (mN/m). In this study a bubble was formed to a maximal radius and 15 seconds later pulsation was started. A continuous tracing of pressure differences was made 1 min after bubble formation.

There were three groups of samples for surface activity measurements. In the first group (n=5) the sample chamber of the surfactometer contained surfactant only. In the second group (n=5) surfactant was mixed with an unrelated mouse-IgG (vol:vol = 1:1). In the third group (n=5) surfactant was mixed with the undiluted culture supernatant of the C1 hybridoma, containing the monoclonal antibody. For the mixing procedure a Vortex mixer was used for 30 seconds, followed by incubation for 30 min at 37 °C with shaking. The final surfactant concentration was 5 mg phospholipid/ml in all groups. The corresponding antibody concentration was approximately 200 ng/ml in both the second and the third groups. Of these groups surface tension was calculated at minimum radius

after 5 min of pulsation.

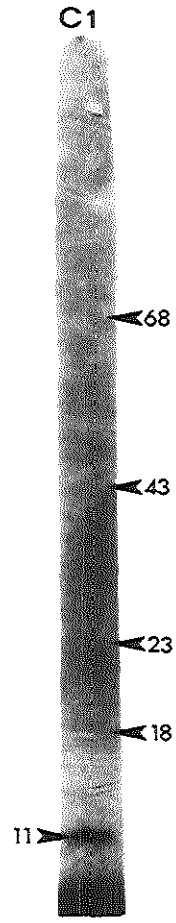


Figure 1. Western blot analysis of antigenic specificity of the C1 monoclonal antibody generated in rats. The procedures used are described in the text. The C1 antibody is directed to an 11 kDa surfactant protein, indicated by the arrow on the left. The markers indicating the positions of proteins with known molecular weight are shown on the right; these are: albumin (68 kDa), ovalbumin (43 kDa), chymotrypsinogen (23 kDa) and RNase (18 kDa).

Animal model

The studies were performed in 28 male adult Wistar rats (body weight: 240-260 g), divided into four groups. Respiratory failure was induced by lung lavage as described by Lachmann et al [20]. In brief: all rats were anesthetized with pentobarbital sodium (60 mg/kg, i.p.), tracheotomized and paralyzed with pancuronium bromide (0.5 mg/kg, i.m.). A catheter was inserted into the carotid artery. Lungs were lavaged six times with 8 ml

warm saline (37°C) to produce a PaO_2<80 mmHg at the following ventilator settings: pressure-controlled ventilation, FiO_2=1.0, ventilation frequency=30/min, peak airway pressure=26 cmH_2O, PEEP=6 cmH_2O and inspiratory/expiratory ratio = 1:2. These ventilator settings were maintained throughout the observation period; a Servo Ventilator 900 C (Siemens-Elema, Solna, Sweden) was used.

Treatment began approximately 5 min after the last lavage. Surfactant was intratracheally instilled in three of four groups at a concentration of approximately 150 mg phospholipids/kg. Group 1 (n=6) received 2 ml surfactant only; group 2 (n=7) received 2 ml surfactant mixed with the monoclonal antibody (C1) (vol:vol = 1:1); group 3 (n=6) received 2 ml surfactant mixed with the control preparation (RPMI-1640 + 10% horse serum in which YB2/0 cells alone had been cultured). Group 4 (n=9) received 2 ml saline only, intratracheally. The antibody in group 2 was used as a culture supernatant at concentrations of 400-500 ng/ml in RPMI-1640 + 10% normal horse serum. For groups 2 and 3, surfactant plus culture supernatant or surfactant plus control preparation, were incubated together for 30 min at 37°C with shaking, as for the measurement of surfactant tension described above.

Blood samples were taken from the carotid artery of each rat just before and 5 min after broncholavage (directly followed by treatment) and at 5, 30, 60, 120 and 180 min post treatment. PaO_2 and $PaCO_2$ were measured with the ABL 330 Acid-Base Laboratory (Radiometer, Copenhagen, Denmark).

Histological examination

At the end of the animal study the rats were sacrificed with an overdose of intra-arterial administered pentobarbital. The lungs were inflated to 20 cm H_2O with air, the trachea was clamped and the lungs removed and fixed in 10% formalin for light microscopy examination. The tissues were dehydrated and embedded in paraffin, and 6 μm sections of the base of the right lung were stained with hematoxylin and eosin.

Stastistical analysis

Statistical evaluation of collected data was performed using the Mann-Whitney-Wilcoxon test. Statistical significance was accepted at $p<0.05$ (two-tailed).

RESULTS

Measurement of surface activity

After 5 min of pulsation, minimum surface tension was high in the surfactant plus C1 sample (17.7 ± 0.63 mN/m), whereas the surfactant alone (4.3 ± 0.76 mN/m) and surfactant plus mouse-IgG (3.1 ± 0.67 mN/m) samples had significantly lower minimum surface tensions; the difference between these latter sample groups was not significant.

Animal studies

Figure 2 shows PaO_2 values for each group. Before lavage PaO_2 values are high in all groups, whereas after lavage all values are low; the intergroup differences both before and after lavage are not statistically significant. After treatment, the difference in PaO_2 values between the surfactant plus C1 group and surfactant alone group remains highly significant throughout the observation period, as does the difference between the surfactant plus C1 group and surfactant plus RPMI-1640 group. The PaO_2 values of the surfactant only group are higher than those of the surfactant plus RPMI-1640 group, but the difference is not significant. Comparing PaO_2 values of the surfactant plus C1 group with the saline group revealed almost identical values after 60 min post-treatment.

Table 1 shows $PaCO_2$ values for all groups; the intergroup differences before and 5 min after lavage are not significant. The $PaCO_2$ values of the group receiving surfactant plus C1 are significantly higher at 60 - 180 min compared to the group receiving surfactant alone. The difference in values between the groups receiving surfactant plus C1 or surfactant plus RPMI-1640 is significant at 60 and 120 min post treatment. As with PaO_2, the difference in $PaCO_2$ values between the surfactant plus RPMI-1640 and surfactant alone groups is not significant at any time. Similarly, there is no significant difference between post lavage $PaCO_2$ values in the surfactant plus C1 group, compared to the saline alone group.

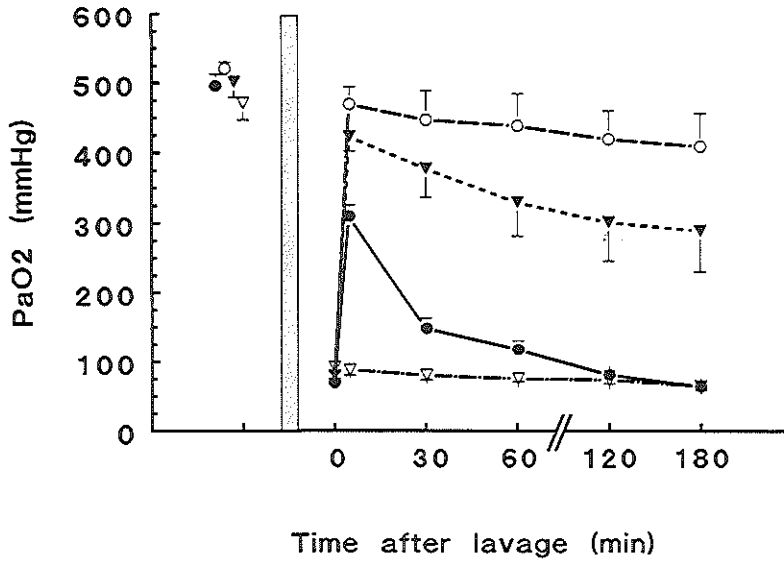


Figure 2. PaO_2 values in mmHg (mean ± SEM) of the four treatment groups: surfactant plus C1 (●); surfactant only (○); surfactant plus RPMI-1640 (▾); saline only (▽). The vertical grey bar indicates the moment of broncholavage; t = 0 indicates PaO_2 values 5 min after lavage, immediately followed by treatment.

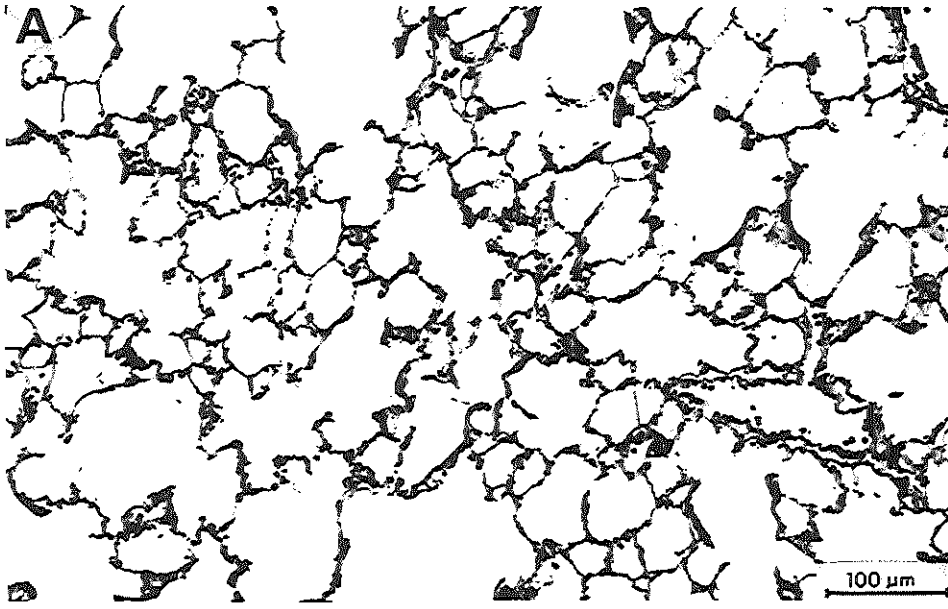


Figure 3. Typical histologic findings in rats: (A) after treatment with surfactant plus RPMI-1640.

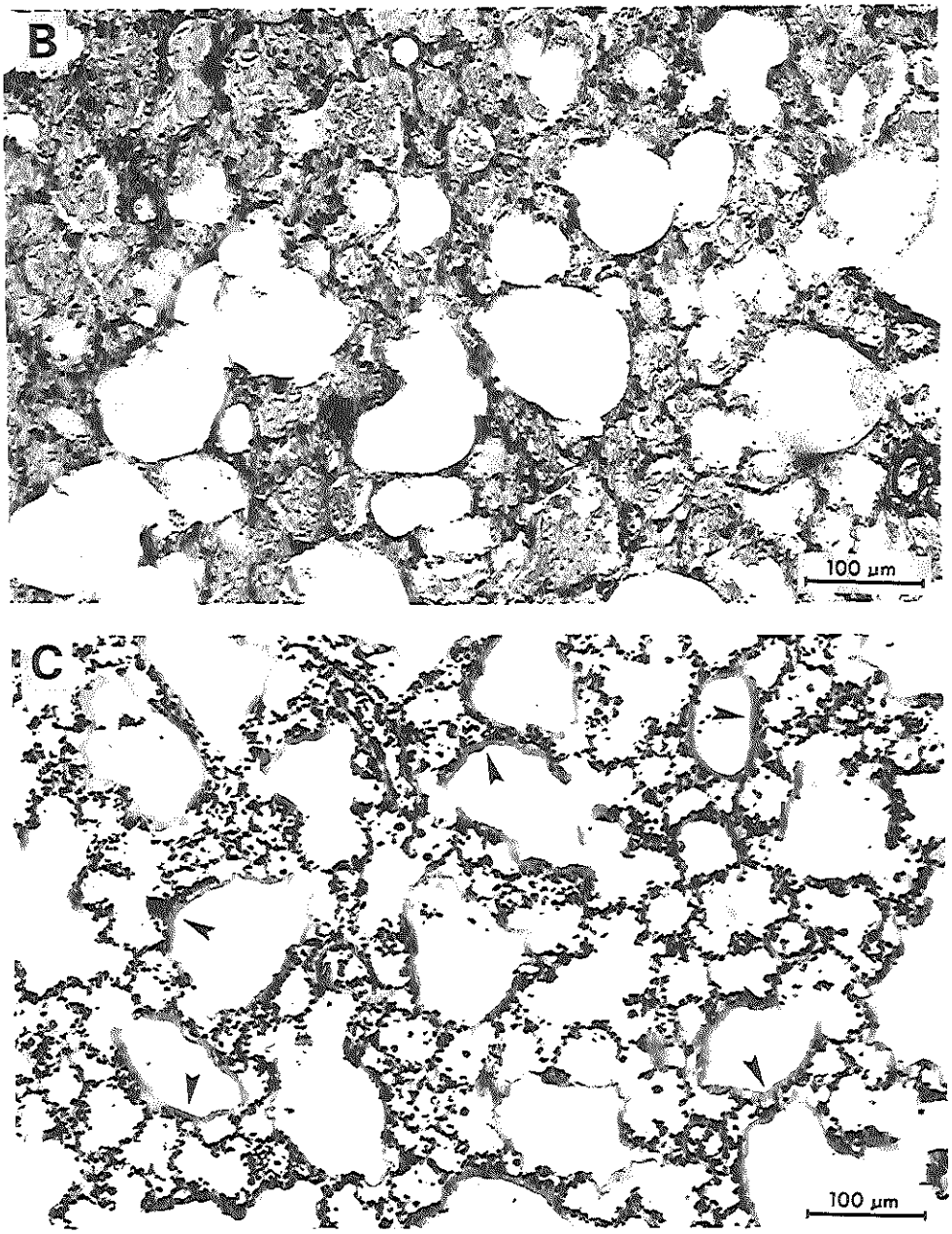


Figure 3. Typical histologic findings in rats: (B) after treatment with saline; (C) after treatment with surfactant plus C1 (arrows indicate the eosinophilic substance covering the alveolar walls). Hematoxylin and eosin stain.

Table 1: $PaCO_2$ values (mmHg) before and after lavage and after different therapeutic regimes.

	Surfactant (n=6)	Surf. + C1 (n=7)	Surf. + RPMI-1640 (n=6)	Saline (n=9)
Before lavage	37.6 ± 1.84	34.7 ± 1.60	39.0 ± 2.10	39.4 ± 3.30
5 min after lavage	57.1 ± 5.76	59.7 ± 2.85	56.2 ± 4.81	51.3 ± 3.77
5 min after treatment	44.3 ± 3.08	50.1 ± 2.30	46.1 ± 1.79	51.5 ± 3.20
30 min	42.7 ± 3.41	46.6 ± 2.41	40.5 ± 1.69	51.8 ± 3.62*
60 min	37.5 ± 2.82	45.6 ± 1.93*	39.2 ± 1.55	52.0 ± 3.79*
120 min	37.7 ± 1.97	47.8 ± 1.98*	39.3 ± 2.48	51.8 ± 3.98*
180 min	39.9 ± 2.99	51.4 ± 3.35*	45.0 ± 3.71	56.6 ± 4.19*

All data are mean ± SEM; *statistically significant difference between surfactant and the other groups; for further significant differences see text.

Light-microscopic findings

The lungs of the animals receiving surfactant only or surfactant plus RPMI-1640 were well aerated (Fig. 3A). The lungs from animals receiving saline showed widespread atelectasis, alternating with areas of hyperexpansion; the alveoli were filled with amorphous exudate and erythrocytes, indicating damage to the alveolar-capillary memrane (Fig. 3B). Lungs of rats which got surfactant plus C1 showed regions with atelectasis, alternating with aerated areas, which were partly overdistended. Especially these overdistended alveoli were covered with an eosinophilic substance. A typical light-microscopic picture is shown in Figure 3C.

DISCUSSION

It has been reported that in adult animal models (guinea pigs, rabbits, pigs and dogs) repeated bilateral whole-lung lavage with saline severely impairs lung function through loss of lung surfactant [20]. It is established that this lung lavage model is useful for a variety of experimental purposes, including e.g. testing of different surfactant preparations, and to demonstrate that exogenous surfactant restores blood gases to normal [21,22,23,24]. Since the purpose of this study was to investigate the influence of a monoclonal antibody on the function of exogenous surfactant containing the hydrophobic surfactant proteins (SP-B and SP-C), the lung lavage model was used to detect any inhibiting effect of this monoclonal antibody on the effectiveness of the exogenous surfactant preparation to restore blood gases. The monoclonal antibody used in this study, C1, recognizes an 11 kDa surfactant associated protein. Tanaka and colleagues have demonstrated that bovine surfactant contains SP-B with a molecular weight of 10 kDa in the reduced state [25]. SP-C has a much lower molecular weight after reduction (for review, See [2]). These results and the findings reported by others suggest that the 11kDa protein is most likely a bovine SP-B monomer.

This C1 monoclonal antibody inhibits pulmonary surfactant function, as demonstrated by low PaO_2 and high surface tension in this study. A similar elevation of the minimum surface tension of porcine surfactant after mixing with a monoclonal antibody against porcine SP-B has also been reported by Kobayashi and colleagues. They also demonstrated that this mixture of porcine surfactant and monoclonal antibody, unlike porcine surfactant alone, is not able to improve lung compliance in immature rabbit fetuses [26]. The reason for this inhibition of surfactant function could be that the monoclonal antibody simply inhibits the function of SP-B. As already mentioned in the introduction, the hydrophobic proteins SP-B and SP-C play an essential role in the function of surfactant [4,5,6].

Another mechanism by which the antibody perhaps inhibits surfactant may lie in the formation of surfactant-antibody aggregates, with subsequent loss of surfactant function. Comparable results have been reported in an in vitro study using IgG subfragments where it was found that surfactant mixed with polyclonal rabbit anti-surfactant antiserum inhibited the ability of surfactant to lower surface tension, as

measured by the pulsating bubble surfactometer. It was demonstrated that bivalent antibody ($F[ab']_2$) preparations inhibited surfactant activity, whereas monovalent antibody (F[ab']) fragments did not [9]. Thus, it was concluded that these polyclonal antibodies agglutinate surfactant. It is possible that this aggregation of surfactant with the C1 monoclonal antibody alters the surface activity of surfactant and/or that the aggregates themselves are responsible for further deterioration of pulmonary function.

Another explanation for the failure of surfactant mixed with the monoclonal antibody to restore lung function can be that the antibody causes a configuration change of the 11 kDa protein, which affects the protein-phospholipid interaction. Also, it is possible that other physicochemical characteristics of surfactant, such as charge, are altered. These changes in characteristics of surfactant could affect the function of surfactant, i.e. surface tension lowering capacity.

The reason for the initial increase of PaO_2 after administration of surfactant plus the monoclonal antibody followed by a decrease of PaO_2 (Fig. 2), could be explained as a functional surfactant deficit. In a recent study (unpublished observations), using the same lung lavage model, the effect of different doses of surfactant, ranging from 10-300 mg/kg, to improve arterial oxygenation was investigated. In adult rats receiving only 10-15 mg surfactant-phospholipids/kg surfactant intratracheally, a similar behavior as observed in the present study could be demonstrated, namely, an initial rise of PaO_2, rapidly followed by deterioration of lung function. Rats receiving the same dose of surfactant used in the present study (150 mg surfactant-phospholipids/kg) showed the same results as the rats receiving surfactant alone in this study, i.e restoration of blood gases to normal values and remaining constantly high for the observed period. From these findings one could conclude that about 90% of the exogenous surfactant was inhibited by the monoclonal antibody and perhaps, in part, by the horse serum. The amount of surfactant which was not inhibited by the monoclonal antibody was too low to stabilize the lungs at the applied ventilator settings for the observed period.

As can be seen in Figure 2, the PaO_2 values of rats receiving surfactant plus control serum (RPMI-1640 + 10% horse serum) are lower than PaO_2 values of rats receiving surfactant alone, though this difference is not significant. An explanation for the lower PaO_2 values in the group receiving surfactant plus control serum could lie in the fact that proteins in the control serum inhibited a part of the exogenous surfactant.

This inhibitory capacity of different serum proteins has already been demonstrated in in vitro studies [27,28].

Histologically, lungs of rats receiving surfactant plus the monoclonal antibody showed an eosinophilic substance covering the alveolar walls. This finding could suggest that the C1 antibody aggregates surfactant by crosslinking 11 kDa surfactant proteins, supporting the suggestion that antibody inactivates surfactant by causing aggregation of surfactant-antibody complexes. On the other hand, it is possible that the histological findings reflect the presence of hyaline memranes due to fibrin deposition, caused by leakage of the alveolar-capillary membrane as a result of a functional surfactant deficit. No experiments were performed in this study to demonstrate the presence of fibrin in the material covering the alveolar walls; but in earlier histological studies hyaline membranes were observed in animal lungs following lung lavage and ventilation for a period of 2-6 h [20,23]. The lungs of rats receiving saline showed widespread atelectasis and edema-filled alveoli. The reason for the difference between the lungs of rats receiving surfactant plus C1 and lungs of rats receiving saline alone could perhaps be explained by the initial protective effect of surfactant in the first group. Perhaps there was still some partial protective effect from surfactant remaining, preventing excessive intra-alveolar edema accumulation, although this effect was not enough to permanently increase PaO_2. When compared to lungs of rats receiving surfactant plus RPMI-1640, lungs of rats receiving surfactant plus C1 showed regions with atelectasis and an eosinophilic substance covering the aerated alveoli.

In conclusion, we have demonstrated that a monoclonal anti-surfactant antibody (C1) to an 11 kDa apoprotein of bovine surfactant (SP-B) inhibits the function of exogenous bovine surfactant, both in vivo and in vitro. As already suggested in other studies [4,5,6], these results confirm the importance of the hydrophobic surfactant proteins for optimal surfactant function in the adult lung.

REFERENCES

1. Hawgood S. - Pulmonary surfactant apoproteins: a review of protein and genomic structure. Am J Physiol 1989; 257: L13-L22.

2. Possmayer F. - A proposed nomenclature for pulmonary surfactant-associated proteins. Am Rev Respir Dis 1988; 138: 990-998.

3. Persson A, Chang D, Rust K, Moxley M, Longmore W, Crouch E. - Purification and biochemical characterization of CP4 (SP-D), a collagenous surfactant-associated protein. Biochemistry 1989; 28: 6361-6367.

4. Revak SD, Merritt TA, Degryse E, Stefani L, Courtney M, Hallman M, Cochrane CG. - Use of human surfactant low molecular weight apoproteins in the reconstitution of surfactant biologic activity. J Clin Invest 1988; 81: 826-833.

5. Yu S-H, Possmayer F. - Reconstitution of surfactant activity using the 6-K apoprotein associated with pulmonary surfactant. Biochem J 1986; 236: 85-89.

6. Whitsett JA, Ohning BL, Ross G, Meuth J, Weaver T, Holm BA, Shapiro DL, Notter RH. - Hydrophobic surfactant-associated protein in whole lung surfactant and its importance for biophysical activity in lung surfactant extracts used for replacement therapy. Pediatr Res 1986; 20: 460-467.

7. Merritt TA, Strayer DS, Hallman M, Spragg RD, Wozniak P. - Immunologic consequences of exogenous surfactant administration. Semin Perinatol 1988; 12: 221-230.

8. Strayer DS, Merritt TA, Spragg R, Hallman M. - Immunogenicity of surfactant and its implications for replacement therapy. In: Surfactant replacement therapy in neonatal and adult respiratory distress syndrome. Ed, Lachmann B, 1988, Berlin, Heidelberg, Springer-Verlag, pp 301-313.

9. Strayer DS, Merritt TA, Makunike C, Hallman M. - Antigenicity of low molecular weight surfactant species. Am J Pathol 1989; 134: 723-732.

10. Strayer DS, Hallman M, Merritt TA. - Immunogenicity of surfactant: I. Human alveolar surfactant. Clin Exp Immunol 1991; 83: 35-40.

11. Metcalfe IL, Enhorning G, Possmayer F. - Pulmonary surfactant-associated proteins: their role in the expression of surface activity. J Appl Physiol 1980; 49: 34-41.

12. Strayer DS, Merritt TA, Lwebuga-Musaka J, Hallman M. - Surfactant-anti-surfactant immune complexes in infants with respiratory distress syndrome. Am J Pathol 1986; 122: 353-362.

13. Kennet RH. - Fusion protocols. In: Monoclonal antibodies. Eds, Kennet RH, McKearn TJ, Bechtol KB, 1980, New York, Plenum Press, pp 365-367.

14. McKearn TJ. - Cloning of hybridoma cells by limiting dilution in fluid phase. In: Monoclonal antibodies. Eds, Kennet RH, McKearn TJ, Bechtol KB, 1980, New York, Plenum Press, p 374.

15. Jonak ZL. - Isolation of monoclonal antibodies from supernatant by $(NH_4)_2SO_4$ precipitation. In: Monoclonal antibodies. Eds, Kennet RH, McKearn TJ, Bechtol KB, 1980, New York, Plenum Press, pp 405-406.

16. Engvall E, Pesce A (eds). - Quantitative enzyme immunoassay. Scand J Immunol 1978; 8 (Suppl 7).

17. Maizel JV, Jr. - Gel electrophoresis of proteins. In: Methods in virology, vol. 5. Eds, Maramorosch K, Koprowski H, 1971, New York, Academic Press, pp 179-246.

18. Towbin H, Staheiln T, Gordon J. - Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979; 76: 4350-4354.

19. Enhorning G. - Pulsating bubble technique for evaluating pulmonary surfactant. J Appl Physiol 1977; 43: 198-203.

20. Lachmann B, Robertson B, Vogel J. - In vivo lung lavage as an experimental model of the respiratory distress syndrome. Acta Anaesthesiol Scand 1980; 24: 231-236.

21. Lachmann B, Fujiwara T, Chida S, Morita T, Konishi M, Nakamura K, Maeta H. - Improved gas exchange after tracheal instillation of surfactant in the experimental adult respiratory distress syndrome. Crit Care Med 1981; 9: 158.

22. Lachmann B, Fujiwara T, Chida S, Morita T, Konishi M, Nakamura K, Maeta H. - Surfactant replacement therapy in the experimental adult respiratory distress syndrome (ARDS). In: Pulmonary surfactant system. Eds, Cosmi EV, Scarpelli EM, 1983, Amsterdam, Elsevier Science Publishers, pp 231-235.

23. Berggren P, Lachmann B, Curstedt T, Grossmann G, Robertson B. - Gas exchange and lung morphology after surfactant replacement in experimental adult respiratory distress syndrome induced by repeated lung lavage. Acta Anaesthesiol Scand 1986; 30: 321-328.

24. Kobayashi T, Kataoka H, Ueda T, Murakami S, Takada Y, Kokubo M. - Effects of surfactant supplement and end-expiratory pressure in lung lavaged rabbits. J Appl Physiol 1984; 57: 995-1001.

25. Tanaka Y, Takei T, Kanazawa Y. - Lung surfactants. II. Effects of fatty acids, triacylglycerols and protein on the activity of lung surfactant. Chem Pharm Bull (Tokyo) 1983; 31: 4100-4109.

26. Kobayashi T, Nitta K, Takahashi R, Kurashima K, Robertson B, Suzuki Y. - Activity of pulmonary surfactant after blocking SP-A and SP-B. J Appl Physiol 1991; 71: 530-536.

27. Fuchimukai T, Fujiwara T, Takahashi A, Enhorning G. - Artificial pulmonary surfactant inhibited by proteins. J Appl Physiol 1987; 62: 429-437.

28. Holm BA, Enhorning G, Notter RH. - A biophysical mechanism by which plasma proteins inhibit lung surfactant activity. Chem Phys Lipids 1988; 49: 49-55.

APPENDIX A

Tables 1 and 2 contain the data of the clinical studies on exogenous surfactant therapy in neonates performed until the end of 1989. These tables are incorporated in a review article on surfactant function, which has been published recently (see Chapter 1).

The major clinical trials performed from begin 1990 until end 1992 are summarized in Tables 3 and 4. These tables contain studies on prophylactic surfactant treatment and on rescue surfactant treatment in neonates, respectively. The references from Tables 3 and 4 are listed at the end of this Appendix.

Table 1: Prophylactic surfactant treatment

Type of surfactant	Source	No. of patients	No. of controls	Treatment criteria	Outcome
Surfactant TA (supplemented bovine homogenate)	Soll et al, 1988 (72)	79	81	Birth weight 750-1250 g, intubated at 4-37 min after birth	FiO_2 ↓; X-chest improved; patients who weighed 750-999 g responded better
Bovine lung lavage extract	Enhorning et al, 1985 (11)	39	33	<30 wk gestation	Incidence of death ↓; incidence of IVH ↓; incidence of air leaks ↓
Calf lung surfactant extract (CLSE)	Shapiro et al, 1985 (67)	16	16	25-29 wk gestation	Severity of RDS decreased transiently
	Wood et al, 1987 (80)	25	35 (saline) 20 (air)	25-29 wk gestation	Incidence of RDS ↓; X-chest shows decreased severity of RDS in treated group
	Kendig et al, 1988 (32)	34	31	25-29 wk gestation	Severity of RDS ↓; incidence of air leaks ↓
	Shennan et al, 1988 (69)	39 (phase 1) 29 (phase 2)	33 27	<30 wk gestation	(See Enhorning, 1985); Both phases: incidence and severity of RDS ↓; PIE ↓; FiO_2 ↓; a/A PO_2 ↑; incidence of BPD ↓ Phase 2: no decrease in IVH and mortality
		16	17	30-33 wk gestation	No effect
Infasurf (CLSE)	Kwong et al, 1985 (36)	14	13	24-28 wk gestation	Severity of RDS at 48 h ↓;FiO_2 ↓; ventilatory support ↓

Table 1 (continued):

Type of surfactant	Source	No. of patients	No. of controls	Treatment criteria	Outcome
	Kwong et al, 1988 (37)	315	0	23-29 wk gestation	Incidence of death ↓
	Egan et al, 1988 (9)	1437 (262 were treated too late)	0	<33 wk gestation	Severity of RDS was higher in patients who were treated too late; mortality ↓ compared to earlier studies
	Shapiro et al, 1988 (68)	78 (prophylaxis) 81 (rescue)	0	24-29 wk gestation; RDS: FiO_2> 0.4, MAP≥ 7cmH_2O, X-chest showing RDS	Both groups: identical mortality; no difference in incidence of complications
	Bloom et al, 1987*	43 (Infasurf) 13 (Human amniotic fluid)	30	24-31 wk gestation	Incidence of death ↓; incidence of PIE ↓; FiO_2 ↓
	Bloom et al, 1988 (3)	30 (Infasurf) 10 (Human amniotic fluid)	30	24-31 wk gestation	Mortality ↓; BPD ↓; PIE ↓; incidence of pneumothorax ↓; FiO_2: CLSE< HAF; MAP: CLSE< HAF; a/A PO_2: CLSE> HAF
Human amniotic fluid	Merritt et al, 1986 (51)	31	29	24-29 wk gestation; L/S ratio≤ 2.0	Incidence of death ↓; incidence of BPD ↓; incidence of air leaks ↓
	Merritt et al, 1988b (53)	23 (prophylaxis) 23 (rescue)	0	24-29 wk gestation; 23 twin pairs: first half: prophylaxis second half: rescue	<6 h after treatment: blood gas values better in prophylaxis group; >6 h: MAP ↓ and FiO_2 ↓ in rescue group

Table 1 (continued):

Type of surfactant	Source	No. of patients	No. of controls	Treatment criteria	Outcome
Synthetic phospholipids (ALEC)	Morley et al, 1981 (54)	22	33	≤34 wk gestation	Incidence of death ↓
	Wilkinson et al, 1982*	16	16	≤32 wk gestation	No effect
	Milner et al, 1984*	10	6	≤34 wk gestation	No effect
	Morley et al, 1985*	243	220	25-34 wk gestation	Incidence of death ↓ before 30 wk
	Ten Centre Study Group, 1987 (55)	159	149	25-29 wk gestation	Mortality ↓; incidence of IVH ↓; hours of ventilation ↓
	Morley et al, 1988 (56)	163	164	23-34 wk gestation	Before 30 wk gestation: incidence of death ↓; IVH ↓; dependence on O_2 therapy ↓
Exosurf (synthetic surfactant)	Phibbs et al, 1988 (61)	29 (prophylaxis)	32	≤34 wk gestation; 700-1350 g birth weight	No effect on survival; FiO_2 ↓ and PIP ↓ during first 72 h
		45 (rescue)	40	>650 g birth weight; RDS: $FiO_2 \geq 0.4$, MAP≥ 7cmH_2O, X-chest showing RDS	Treated group shows higher survival; FiO_2 ↓ during first 48 h
DPPC + HDL	Halliday et al, 1984 (21)	49	51	25-33 wk gestation	No effect

a/A PO_2 = arterial-alveolar PO_2 ratio; ALEC = Artificial Lung Expanding Compound; BPD = bronchopulmonary dysplasia; HAF = Human amniotic fluid; HMD = hyaline membrane disease; IVH = intraventricular hemorrhage; MAP = mean airway pressure; PDA = patent ductus arteriosus; PIE = pulmonary interstitial emphysema; PIP = positive inspiratory pressure; ↑ = increase; ↓ = decrease; *cited from (52)

Table 2: Rescue surfactant treatment

Type of surfactant	Source	No. of patients	No. of controls	Treatment criteria	Outcome
Surfactant TA	Fujiwara et al, 1980 (13)	10	0	RDS	PaO_2 ↑; $PaCO_2$ ↓; a/A PO_2 ↑; 9 patients had PDA
	Fujiwara et al, 1981 (14)	19	0	RDS	PaO_2/FiO_2 ↑; FiO_2 ↓; incidence of PDA ↑
	Fujiwara et al, 1984*	20	10	RDS	a/A PO_2 ↑; MAP ↓; X-chest improved;
	Fujiwara et al, 1984 (15)	37	5	RDS	a/A PO_2 ↑; FiO_2 ↓; X-chest improved; incidence of PDA ↑
	Fujiwara et al, 1987 (16)	91	yes	RDS	Early improvement; IVH ↓; BPD ↓; incidence of death ↓; pneumothorax ↓
	Raju et al, 1987 (62)	17	13	HMD; birth weight 750-1750 g; age≤ 6 h; MAP> 8cmH_2O; a/A PO_2≤ 0.24	a/A PO_2 ↑; FiO_2 ↓; incidence of PDA ↓; BPD ↓; air leaks ↓
	Gitlin et al, 1987 (18)	18	23	Age< 8 h; birth weight≥ 1000 g; FiO_2≥ 0.4	a/A PO_2 ↑; MAP ↓; time on ventilator ↓; no. of days with FiO_2≥ 0.4 ↓
	Fujiwara et al, 1988 (17)	96	0	Birth weight 520-2400 g; MAP> 7cmH_2O; FiO_2> 0.4	"Pure RDS": sustained improvement "RDS + PDA": at first improvement; secondly relapse; after treatment of PDA: recovery

Table 2 (continued):

Type of surfactant	Source	No. of patients	No. of controls	Treatment criteria	Outcome
	Horbar et al, 1988b (28)	38	46	Birth weight 750-1750 g	a/A PO_2 ↑; MAP ↓; incidence of IVH ↑
	Horbar et al, 1988a (27)	78	81	Birth weight 750-1750 g	FiO_2 ↓; MAP ↓; a/A PO_2 ↑; incidence of ventilatory support > 48 h ↓
	Konishi et al, 1988b (35)	206	0	23-39 wk gestation; birth weight 600-3400 g; MAP> 7cmH_2O; FiO_2> 0.4	Group I (good response): mortality 10%; FiO_2 ↓; MAP ↓; a/A PO_2 ↑ Group II (poor response): mortality 22%; no increase in a/A PO_2
	Konishi et al, 1988a (34)	23 (high dose) 23 (low dose)	0	Birth weight 1000-1500 g	Both groups a/A PO_2↑; High dose: prolonged effect of treatment; lower incidence of IVH and BPD; lower no. of patients treated with O_2 for 30 days
	Charon et al, 1989 (4)	29	23	Age< 8 h; birth weight 750-1750 g	(See Gitlin, 1987); in the lasting response group the a/A PO_2 improved > 48 h
Bovine lung lavage extract	Smyth et al, 1981 (70)	3	0	Severe RDS	PaO_2 ↑; FiO_2 ↓; X-chest improved

Table 2 (continued):

Type of surfactant	Source	No. of patients	No. of controls	Treatment criteria	Outcome
	Smyth et al, 1983 (71)	6	0	Severe RDS	a/A PO_2 ↑ (24 h effect)
	Shennan et al, 1988 (69)	22 (1 dose) 17 (≥ 1 dose) (phase 3)	20	30-36 wk gestation	Prolonged improvement of gas exchange in group receiving ≥ 1 dose; no difference in mortality and morbidity between treatment groups and control group
SF-RI 1 (bovine surfactant)	Gortner et al, 1988 (19)	15 (high dose) 19 (low dose)	0	24-31 wk gestation; birth weight 430-1500 g; FiO_2> 0.5	High dose: survival ↑; both groups: FiO_2 ↓; MAP ↓; a/A PO_2 ↑
CLSE	Davis et al, 1988 (7)	35	0	FiO_2≥ 0.4	Improvement of gas exchange; improvement of lung mechanics only during spontaneous breathing
Bovine or porcine surfactant	Mortensson et al, 1987 (57)	10	0	27-32 wk gestation; birth weight 795-1680 g	X-chest: improvement of aeration, distension of bronchioli, right-left shunt ↓
	Noack et al, 1987 (58)	10	0	See Mortensson, 1987	a/A PO_2 ↑
Porcine surfactant	Svenningsen et al, 1987 (74)	4	4	Birth weight 700-1400 g; FiO_2> 0.6	Transcutaneous PO_2 ↑; X-chest improved
	McCord et al, 1988b (49)	12	8	FiO_2> 0.6	Incidence of IVH ↓ and pneumothorax ↓

Table 2 (continued):

Type of surfactant	Source	No. of patients	No. of controls	Treatment criteria	Outcome
	McCord et al, 1988a (48)	14	15	Birth weight < 2000 g; FiO_2 > 0.6	a/A PO_2 ↑; incidence of IVH ↓ and pneumothorax ↓
Porcine CK	Kobayashi et al, 1981*	1	0	RDS	PaO_2/FiO_2 ↑; $PaCO_2$ ↓; X-chest improved
	Ohta et al, 1981*	1	0	RDS	"Improvement"
	Nohara et al, 1983*	6	0	RDS	PaO_2 ↑ in 4 patients; X-chest improved
Curosurf (porcine surfactant)	Berggren et al, 1984*	4	0	Severe RDS	PaO_2 ↑; $PaCO_2$ ↓; incidence of air leaks ↓
	Robertson et al, 1988a (64)	77	69	Birth weight 700-2000 g	Incidence of death ↓; BPD ↓
	Robertson et al, 1988b (65)	55	0	Birth weight 700-2000 g	Incidence of death ↓; BPD ↓
	Speer et al, 1988 (73)	14	20	Severe RDS	Oxygenation ↑; duration of PIP and FiO_2 > 0.4 ↓; mortality ↓; PDA ↑; pneumothorax ↓; no difference in BPD and IVH
Human amniotic fluid	Hallman et al, 1982 (22)	3	0	RDS	PaO_2 ↑; FiO_2 ↓; MAP ↓

Table 2 (continued):

Type of surfactant	Source	No. of patients	No. of controls	Treatment criteria	Outcome
	Hallman et al, 1983 (23)	5	5	Mean birth weight 974 ± 61 g	PaO_2 ↓; $PaCO_2$ ↓; pH ↑; X-chest improved; severity of RDS ↓
	Merritt et al, 1983 (50)	4	3	Severe RDS	PaO_2 ↑; $PaCO_2$ ↓; FiO_2 ↓; MAP ↑; X-chest impoved
	Hallman et al, 1985 (24)	25	28	Age< 10 h; birth weight< 1500 g; L/S ratio≤ 2.0	Incidence of death ↓; BPD ↓; incidence of air leaks ↓; no. of patients requiring FiO_2> 0.3 at 30 days ↓
	Heldt et al, 1988 (26)	49	12	25-29 wk gestation; birth weight 450-1580 g	Maturity of ductus arteriosus parallels maturity of the lung and is independent of surfactant therapy
	Lang et al, 1988 (45)	10	14	25-32 wk gestation; mean treatment time: 5.6 h	PaO_2 ↑; FiO_2 ↓; a/A PO_2 ↑; MAP ↓
ALEC	Milner et al, 1983*	10	0	RDS	No effect
	Weintraub et al, 1985*	22	0	FiO_2> 0.6; PIP≥ 20cmH_2O	$PaCO_2$ ↓; PIP ↓; 8 patients died
	Wilkinson et al, 1985*	12	0	<32 wk gestation	No effect; L/S ratio ↑ in tracheal fluid

Table 2 (continued):

Type of surfactant	Source	No. of patients	No. of controls	Treatment criteria	Outcome
	Wilkinson et al, 1985 (79)	16 (trial I) 12 (trial II)	16 12	<32 wk gestation; L/S ratio immature	No significant difference in ventilation pressures or O_2 therapy used, nor in mortality and morbidity< 2 years
Liposomal phospholipids	Obladen et al, 1988 (59)	10	0	<30 wk gestation; birth weight< 1500 g	a/A PO_2 ↑; FiO_2 ↓; lung compliance ↑; 3 patients died
Artificial surfactant	Friedman et al, 1982 (12)	3	0	RDS	X-chest improved; "clinical improvement" in 2 patients
Synthetic L-α-lecithin	Robillard et al, 1964 (66)	11	0	RDS	Cyanosis ↓; retraction score ↓
Dipalmitoyl L-α-lecithin	Chu et al, 1967 (5)	27	21	RDS	No beneficial effect

a/A PO_2 = arterial-alveolar PO_2 ratio; ALEC = Artificial Lung Expanding Compound; BPD = bronchopulmonary dysplasia; CLSE = Calf Lung Surfactant Extract; HAF = Human amniotic fluid; HMD = hyaline membrane disease; IVH = intraventricular hemorrhage; MAP = mean airway pressure; PDA = patent ductus arteriosus; PIE = pulmonary interstitial emphysema; PIP = positive inspiratory pressure; ↑ = increase; ↓ = decrease; *cited from (52)

Table 3: Prophylactic surfactant treatment

Type of surfactant	Source	No. of patients	No. of controls	Treatment criteria	Outcome
Survanta (=Surfactant TA)	Hoekstra et al, 1991 (1)	210	220	23-29 wk gestation; birth weight 600-1250 g; treated within 15 min after birth	Mortality ↓; incidence of BPD ↓ and PIE ↓; incidence of RDS ↓; improved oxygenation
	Ferrara et al, 1991 (2)	26	28	Birth weight 600-750 g; treated within 15 min after birth	Mortality ↓; increase of normal survivors compared to control group
Alveofact (=SF-RI 1)	Gortner et al, 1990 (3)	34	35	25-30 wk gestation; mechanical ventilation	Mortality ↓; incidence of BPD ↓; improved oxygenation; MAP ↓; time on mechanical ventilation ↓
Bovine surfactant	Dunn et al, 1991 (4)	62 (prophylaxis) 31 (rescue)	60	<30 wk gestation	Improved oxygenation and lower incidence of air leaks and severe chronic lung disease in surfactant groups; prophylaxis group had higher incidence of mild chronic lung disease compared to rescue group
Infasurf (=CLSE)	Kendig et al, 1991 (5)	235 (prophylaxis) 244 (rescue)	0	<30 wk gestation; Rescue: $FiO_2 \geq 0.4$ and/or MAP≥ 7 cmH_2O	Higher survival, and incidence of pneumothorax ↓ in prophylaxis group, especially in infants delivered at ≤26 wk gestation

Table 3 (continued):

Type of surfactant	Source	No. of patients	No. of controls	Treatment criteria	Outcome
Human amniotic fluid	Merritt et al, 1991 (6)	55 (prophylaxis) 50 (rescue)	52	24-29 wk gestation	Mortality ↓, PIE ↓ and improved indexes of oxygenation in surfactant groups
ALEC	Morley et al, 1991 (7)	102	108	23-34 wk gestation	<29 wk gestation: compliance ↑ from 6-24 h after birth; >29 wk gestation: compliance ↓
Exosurf	Bose et al, 1990 (8)	192	193	Birth weight 700-1350 g	Mortality ↓; survival without BPD ↑; PIE ↓
	Corbet et al, 1991 (9)	224	222	Birth weight 700-1100 g; mechanical ventilation	Mortality ↓; no difference in incidence of RDS; PIE ↓; MAP ↓; O_2 requirements ↓
	Phibbs et al, 1991 (10)	36 (prophylaxis) 53 (rescue)	38 51	Propylaxis: birth weight 700-1350 g Rescue: birth weight >650 g; RDS	FiO_2 ↓ and MAP ↓ during first 72 h in surfactant groups; no difference in IVH and PDA between surfactant groups and controls
	Stevenson et al, 1992 (11)	106	109	Birth weight 500-699 g	O_2 requirements ↓; $P(A\text{-}a)O_2$ improved; MAP ↓; incidence of pneumothorax ↓; death from RDS ↓; overall mortality unchanged; incidence of pulmonary hemorrhage ↑

Table 3 (continued):

Type of surfactant	Source	No. of patients	No. of controls	Treatment criteria	Outcome
Exosurf	The OSIRIS Collaborative Group, 1992 (12)	Part I: 1344 (early) 1346 (delayed) Part II: 3381 (2 doses) 3376 (2-4 doses)	0	Part I: Early: at risk; age< 2h Delayed: age> 2h; RDS; a/A PO_2<0.22 Part II: at risk or RDS	Part I: risk of death or dependence on extra O_2 at expected date of delivery were lower in early treated infants; incidence of pneumothorax ↑ in early treated infants; Part II: no difference in outcome

a/A PO_2 = arterial-alveolar PO_2 ratio; ALEC = Artificial Lung Expanding Compound; BPD = bronchopulmonary dysplasia; IVH = intraventricular hemorrhage; MAP = mean airway pressure; PDA = patent ductus arteriosus; PIE = pulmonary interstitial emphysema; P(A-a)O_2 = alveolar-arterial PO_2 gradient; PIP = positive inspiratory pressure; ↑ = increase; ↓ = decrease

Table 4: Rescue surfactant treatment

Type of surfactant	Source	No. of patients	No. of controls	Treatment criteria	Outcome
Survanta (=Surfactant TA)	Fujiwara et al, 1990 (13)	54	46	Birth weight 750-1749 g	Severity of RDS ↓; incidence of PIE ↓, IVH ↓ and pneumothorax ↓; number of infants surviving without IVH and BPD ↑; no effect on overall mortality
	Couser et al, 1990 (14)	47	47	Birth weight 600-1250 g	Dynamic compliance ↑; oxygenation improved; MAP ↓
	Horbar et al, 1990 (15)	53	53	Birth weight 750-1750 g; $FiO_2 \geq 0.4$	a/A PO_2 ↑; FiO_2 ↓; MAP ↓; no effect on mortality; PIH ↑ in surfactant treated group
	Bhat et al, 1990 (16)	6	9	Age < 6h: 27-32 wk gestation; birth weight 750-1750 g; $FiO_2 \geq 0.4$	Oxygenation improved; MAP ↓; airway resistance ↓; improved dynamic compliance only at 24 h
	Reller et al, 1991 (17)	22	14	>26 wk gestation; birth weight <1750 g; $FiO_2 \geq 0.4$	No effect on PDA
	Liechty et al, 1991 (18)	402	396	Birth weight 600-1750 g	Mortality ↓; incidence of BPD ↓ and PIE ↓; oxygenation improved

Table 4 (continued):

Type of surfactant	Source	No. of patients	No. of controls	Treatment criteria	Outcome
Bovine surfactant	Dunn et al, 1990 (19)	24 (1 dose) 27 (>1 dose)	24	Age< 6 h; 30-36 wk gestation	Improvement of oxygenation in both surfactant groups; multiple doses: sustained improvement of oxygenation
Infasurf (=CLSE)	Auten et al, 1991 (20)	7 (pneumonia) 7 (MAS)	0	Respiratory failure in full-term newborns due to pneumonia or MAS	Improvement of oxygenation; X-chest improved; MAP ↓; no patient died
	Goldsmith et al, 1991 (21)	7	0	Age< 9 h; gestational age: 28.3 ± 2.6 wks; birth weight: 1080 ± 361 g	FRC ↓; specific compliance ↓; specific airway conductance ↓; oxygenation improved
	Davis et al, 1992 (22)	28	0	Respiratory failure due to RDS, pneumonia or MAS, which did not respond to CMV and surfactant therapy	HFJV and ≥1 dose of surfactant improved several respiratory variables
Curosurf	Speer et al, 1990 (23)	19 (early) 7 (late)	0	Severe RDS; $FiO_2 \geq 0.6$; early: 2-15 h of age; late: >15-48 h of age	Improvement of oxygenation; no. of days with $FiO_2 > 0.21$ was increased in late-treatment; incidence of severe BPD increased in late-treatment

Table 4 (continued):

Type of surfactant	Source	No. of patients	No. of controls	Treatment criteria	Outcome
Curosurf	Collaborative European Multicentre Study Group, 1991 (24)	164	69	Birth weight 700-2000 g; $FiO_2 \geq 0.6$	Mortality ↓; incidence of PIE ↓ and pneumothorax ↓; a/A PO_2 ↓; no. of survivors without chronic lung disease ↑
	Speer et al, 1992 (25)	176 (1 dose) 167 (≥1 dose)	0	Birth weight 700-2000 g; $FiO_2 \geq 0.6$	a/A PO_2 ↑ and MAP ↓ in both groups; Mortality and incidence of pneumothorax were lower in the multiple-dose group at 28 days
Human amniotic fluid	Lang et al, 1990 (26)	28	31	24-32 wk gestation; birth weight ≤1500 g	FiO_2 ↓; a/A PO_2 ↑; air leaks ↓; no effect on survival and incidence of BPD
Exosurf	Long et al, 1991 (27)	206 (1-2 doses)	213	Birth weight 700-1350 g; a/A $PO_2 < 0.22$	Mortality ↓; a/A PO_2 ↑; incidence of PIE ↓ and pneumothorax ↓; no effect on BPD
	Long et al, 1991 (28)	614	623	Age < 24 h; birth weight ≥1250 g; a/A $PO_2 < 0.22$	Mortality ↓; incidence of BPD ↓, air leaks ↓, IVH ↓, seizures ↓, PDA ↓, hypotension ↓, pulmonary hypertension ↓; incidence of pulmonary hemorrhage ↑

Table 4 (continued):

Type of surfactant	Source	No. of patients	No. of controls	Treatment criteria	Outcome
Exosurf	Bhutani et al, 1992 (29)	13	19	Birth weight >1100 g	Dynamic compliance ↑ during first 7 days; pulmonary resistance ↓ during first 2 weeks
	Goldman et al, 1992 (30)	20	10	Birth weight 500-1100 g	No difference in lung compliance and pulmonary resistance at 28 days of age
	Hazan et al, 1992 (31)	12	11	Birth weight >1000 g; FiO_2<0.65	No effect on energy expenditure

a/A PO_2 = arterial-alveolar PO_2 ratio; BPD = bronchopulmonary dysplasia; CMV = conventional mechanical ventilation; FRC = functional residual capacity; HFJV = high-frequency jet ventilation; IVH = intraventricular hemorrhage; MAP = mean airway pressure; MAS = meconium aspiration syndrome; PDA = patent ductus arteriosus; PIE = pulmonary interstitial emphysema; PIH = periventricular-intraventricular hemorrhage; PIP = positive inspiratory pressure; ↑ = increase; ↓ = decrease

REFERENCES

1. Hoekstra RE, Jackson JC, Myers TF, Frantz ID 3d, Stern ME, Powers WF, Maurer M, Raye JR, Carrier ST, Gunkel JH, et al. Improved neonatal survival following multiple doses of bovine surfactant in very premature neonates at risk for respiratory distress syndrome. Pediatrics 1991; 88: 10-18
2. Ferrara TB, Hoekstra RE, Couser RJ, Jackson JC, Anderson CL, Myers TF, Raye JR. Effects of surfactant therapy on outcome of infants with birth weights of 600 to 750 grams. J Pediatr 1991; 119: 455-457
3. Gortner L, Bernsau U, Hellwege HH, Hieronimi G, Jorch G, Reiter HL. A multicenter randomized controlled clinical trial of bovine surfactant for prevention of respiratory distress syndrome. Lung 1990; 168 (Suppl): 864-869
4. Dunn MS, Shennan AT, Zayack D, Possmayer F. Bovine surfactant replacement therapy in neonates of less than 30 weeks' gestation: a randomized controlled trial of prophylaxis versus treatment. Pediatrics 1991; 87: 377-386
5. Kendig JW, Notter RH, Cox C, Reubens LJ, Davis JM, Maniscalco WM, Sinkin RA, Bartoletti A, Dweck HS, Horgan MJ, Risemberg H, Phelps DL, Shapiro DL. A comparison of surfactant as immediate prophylaxis and as rescue therapy in newborns of less than 30 weeks' gestation. N Engl J Med 1991; 324: 865-871
6. Merritt TA, Hallman M, Berry C, Pohjavuori M, Edwards DK 3d, Jaaskelainen J, Grafe MR, Vaucher Y, Wozniak P, Heldt G, et al. Randomized, placebo-controlled trial of human surfactant given at birth versus rescue administration in very low birth weight infants with lung immaturity. J Pediatr 1991; 118: 581-594
7. Morley CJ, Greenough A. Respiratory compliance in premature babies treated with artificial surfactant (ALEC). Arch Dis Child 1991; 66: 467-471
8. Bose C, Corbet A, Bose G, Garcia-Prats J, Lombardy L, Wold D, Donlon D, Long W. Improved outcome at 28 days of age for very low birth weight infants treated with a single dose of a synthetic surfactant. J Pediatr 1990; 117: 947-953
9. Corbet A, Bucciarelli R, Goldman S, Mammel M, Wold D, Long W. Decreased mortality rate among small premature infants treated at birth with a single dose of synthetic surfactant: a multicenter controlled trial. J Pediatr 1991; 118: 277-284
10. Phibbs RH, Ballard RA, Clements JA, Heilbron DC, Phibbs CS, Schlueter MA, Sniderman SH, Tooley WH, Wakeley A. Initial clinical trial of EXOSURF, a protein-free synthetic surfactant, for the prophylaxis and early treatment of hyaline membrane disease. Pediatrics 1991; 88: 1-9
11. Stevenson D, Walther F, Long W, Sell M, Pauly T, Gong A, Easa D, Pramanik A, LeBlanc M, Anday E, et al. Controlled trial of a single dose of synthetic surfactant at birth in premature infants weighing 500 to 699 grams. J Pediatr 1992; 120: S3-S12

12. The OSIRIS Collaborative Group. Early versus delayed neonatal administration of a synthetic surfactant - the judgement of OSIRIS. Lancet 1992; 340: 1363-1369
13. Fujiwara T, Konishi M, Chida S, Okuyama K, Ogawa Y, Takeuchi Y, Nishida H, Kito H, Fujimura M, Nakamura H, et al. Surfactant replacement therapy with a single postventilatory dose of a reconstituted bovine surfactant in preterm neonates with respiratory distress syndrome: final analysis of a multicenter, double-blind, randomized trial and comparison with similar trials. Pediatrics 1990; 86: 753-764
14. Couser RJ, Ferrara TB, Ebert J, Hoekstra RE, Fangman JJ. Effects of exogenous surfactant therapy on dynamic compliance during mechanical breathing in preterm infants with hyaline membrane disease. J Pediatr 1990; 116: 119-124
15. Horbar JD, Soll RF, Schachinger H, Kewitz G, Versmold HT, Lindner W, Duc G, Mieth D, Linderkamp O, Zilow EP, et al. A European multicenter randomized controlled trial of single dose surfactant therapy for idiopathic respiratory distress syndrome. Eur J Pediatr 1990; 149: 416-423
16. Bhat R, Dziedzic K, Bhutani VK, Vidyasagar D. Effect of single dose surfactant on pulmonary function. Crit Care Med 1990; 18: 590-595
17. Reller MD, Buffkin DC, Colasurdo MA, Rice MJ, McDonald RW. Ductal patency in neonates with respiratory distress syndrome. A randomized surfactant trial. Am J Dis Child 1991; 145: 1017-1020
18. Liechty EA, Donovan E, Purohit D, Gilhooly J, Feldman B, Noguchi A, Denson SE, Sehgal SS, Gross I, Stevens D, et al. Reduction of neonatal mortality after multiple doses of bovine surfactant in low birth weight neonates with respiratory distress syndrome. Pediatrics 1991; 88: 19-28
19. Dunn MS, Shennan AT, Possmayer F. Single- versus multiple-dose surfactant replacement therapy in neonates of 30 to 36 weeks' gestation with respiratory distress syndrome. Pediatrics 1990; 86: 564-571
20. Auten RL, Notter RH, Kendig JW, Davis JM, Shapiro DL. Surfactant treatment of full-term newborns with respiratory failure. Pediatrics 1991; 87: 101-107
21. Goldsmith LS, Greenspan JS, Rubenstein D, Wolfson MR, Shaffer TH. Immediate improvement in lung volume after exogenous surfactant: alveolar recruitment versus increased distention. J Pediatr 1991; 119: 424-428
22. Davis JM, Richter SE, Kendig JW, Notter RH. High-frequency jet ventilation and surfactant treatment of newborns with severe respiratory failure. Pediatr Pulmonol 1992; 13: 108-112
23. Speer CP, Harms K, Herting E, Neumann N, Curstedt T, Robertson B. Early versus late surfactant replacement therapy in severe respiratory distress syndrome. Lung 1990; 168 (Suppl): 870-876
24. Collaborative European Multicentre Study Group (Robertson B, et al). Factors influencing the clinical response to surfactant replacement therapy in babies with severe respiratory distress syndrome. Eur J Pediatr 1991; 150: 433-439
25. Speer CP, Robertson B, Curstedt T, Halliday HL, Compagnone D, Gefeller O, Harms K, Herting E, McClure G, Reid M, et al. Randomized European multicenter trial of surfactant replacement

therapy for severe neonatal respiratory distress syndrome: single versus multiple doses of Curosurf. Pediatrics 1992; 89: 13-20

26. Lang MJ, Hall RT, Reddy NS, Kurth CG, Merritt TA. A controlled trial of human surfactant replacement therapy for severe respiratory distress syndrome in very low birth weight infants. J Pediatr 1990; 116: 295-300

27. Long W, Thompson T, Sundell H, Schumacher R, Volberg F, Guthrie R. Effects of two rescue doses of a synthetic surfactant on mortality rate and survival without bronchopulmonary dysplasia in 700- to 1350-gram infants with respiratory distress syndrome. J Pediatr 1991; 118: 595-605

28. Long W, Corbet A, Cotton R, Courtney S, McGuiness G, Walter D, Watts J, Smyth J, Bard H, Chernick V. A controlled trial of synthetic surfactant in infants weighing 1250 g or more with respiratory distress syndrome. N Engl J Med 1991; 325: 1696-1703

29. Bhutani VK, Abbasi S, Long WA, Gerdes JS. Pulmonary mechanics and energetics in preterm infants who had respiratory distress syndrome treated with synthetic surfactant. J Pediatr 1992; 120: S18-S24

30. Goldman SL, Bosque E, McCann E, Lewis K. Pulmonary mechanics in premature infants one month after treatment with synthetic surfactant. J Pediatr 1992; 120: S25-S28

31. Hazan J, Chessex P, Piedboeuf B, Bourgeois M, Bard H, Long W. Energy expenditure during synthetic surfactant replacement therapy for neonatal respiratory distress syndrome. J Pediatr 1992; 120: S29-S33

APPENDIX B

Table 2: Case reports

Source	Surfactant	Dosage	Patients	Outcome	Comments
B. Lachmann, 1988 (72)	Bovine surfactant	300 mg/kg	pt.1: Sepsis + pneumonia; PaO_2 = 19 mmHg at pressure controlled ventilation, FiO_2 = 1.0, PAP = 48 cmH_2O, PEEP = 12 cmH_2O and I:E = 3:1	PaO_2↑ (19→240 mmHg), $PaCO_2$↓ (68→45 mmHg); X-chest improved	
P.S. Richman et al, 1989 (91)	Curosurf	4 g (~50-100 mg/kg)	pt.1: Aspiration pneumonitis pt.2: Sepsis pt.3: Pancreatitis All patients intubated <72 h	pts.1+2: Transient (< 1 h) PaO_2↑ pt.3: Persisting (72 h) PaO_2↑	-Unknown ventilator settings -Exact surf. dosage/kg is unknown
Th. Joka et al, 1989 (49)	Bovine surfactant	50 mg/kg (devided in 2 dosages: day 9 and 13)	pt.1: Trauma: ISS = 45 points; PaO_2 = 60 mmHg at FiO_2 = 0.8 and PEEP = 18 cmH_2O; Compliance↓	PaO_2↑, FiO_2↓; Compliance↑; Protein and elastase concentration in BAL↓; Extubated on day 29	No adverse (immunological) reaction to surf.
S. Nosaka et al, 1990 (85)	Surfactant TA	240 mg/instillation	pt.1: 1st episode: Burns; surf. on day 4 and 5; 2nd episode: Aspiration pneumonitis; almost daily surf. between day 13 and 38 pt.2: Post-operative pneumonitis; surf. on days 1, 2 and 3	pt.1: 1st episode: PaO_2↑ at FiO_2 = 1.0 (57→159 mmHg); X-chest improved; 2nd episode: PaO_2↑ at FiO_2 = 0.9 (86→224 mmHg); X-chest improved; pt.2: PaO_2↑ with 144 mmHg; X-chest improved	-Unknown ventilator settings -Exact surf. dosage/kg is unknown -No antibodies to surf. detected

Table 2 (continued):

Source	Surfactant	Dosage	Patients	Outcome	Comments
G. Marraro, 1991 (78)	Curosurf	pt.1: 60 mg/kg pt.2: 40 mg/kg	pt.1: ALL + bone marrow transplant; PaO_2=60 mmHg at FiO_2=1.0 and PEEP=12 cmH_2O pt.2: AML + massive pulmonary hemorrhage	pts. 1+2: PaO_2↑ and $PaCO_2$↓ during first 3 h; Compliance: no effect; X-chest improved	-pts. 1+2: Thromboembolic pathology -Unknown ventilator settings
J. Weg et al, 1991 (112)	Exosurf	Group 1: 6 mg DPPC/kg nebulized during 12 h/day Group 2: 12 mg DPPC/kg nebulized during 24 h/day Group 3: saline nebulized during 12/24 h/day Groups 1-3: treatment during 5 days	Sepsis: Group 1: n=18 Group 2: n=17 Group 3: n=17	Mortality in groups 1-3: 39%, 35% and 47%; Groups 1+2: Compliance↑, Qs/Qt↓	-Unknown ventilator settings -Nebulized surf. -1 pneumothorax in surf. treated group
H. Wiedemann et al, 1992 (114)	Exosurf	Group 1: 40.5 mg DPPC/ml, nebulized during 24 h Group 2: 81 mg DPPC/ml, nebulized during 24 h Group 3: saline nebulized during 24 h Groups 1-3: treatment during 5 days	Sepsis: APACHE II~17 Group 1: n=16 Group 2: n=17 Group 3: n=16	Mortality in groups 1-3: 25%, 35% and 50%; Groups 1+2: $P(A\text{-}a)O_2$ and FiO_2 improved	-Unknown ventilator settings -Nebulized surf. -1 pt. with skin rash and 2 pts. with increased airway pressure in surf. treated groups

Table 2 (continued):

Source	Surfactant	Dosage	Patients	Outcome	Comments
K. Stubbig et al, 1992 (108)	Alveofact	76 mg/kg	pt.1: Trauma: lung contusion, multiple rib fractures and pneumothorax; double-lumen tube: right lung: CMV, FiO_2=0.4, PEEP=5 cmH_2O and I:E=1:1; left lung: high frequency jet ventilation (500/min), FiO_2=1.0; surf. on day 15	Initial deterioration; single-lumen tube, FiO_2=0.8; after removal of crusts via bronchoscopy improvement of gas exchange, FiO_2↓, PEEP↓, I:E=3:1→1:1; X-chest improved	Deterioration after surf. probably due to plugs in airways preventing surf. from sufficiently entering the alveoli
K. Kurashima et al, 1991 (58)	Surfactant TA	10 mg (total amount)	Mild asthmatic attack: Group 1 (n=6): surf. Group 2 (n=5): saline	Group 1: wheezing↓, FVC: 11.7%↑, FEV_1: 27.3%↑, MMF: 33.2%↑, PaO_2: 67→76 mmHg Group 2: no effect	-Asthmatic attack -Spontaneous breathing -Nebulized surf.

ALL = acute lymphatic leukemia; AML = acute myeloid leukemia; BAL = bronchoalveolar lavage; CMV = continuous mechanical ventilation; DPPC = dipalmitoylphosphatidylcholine; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; I:E = inspiratory-expiratory ratio; ISS = injury severity score; MMF = maximal midexpiratory flow; $P(A\text{-}a)O_2$ = alveolar-arterial PO_2 gradient; PAP = peak airway pressure; PEEP = positive end-expiratory pressure; Qs/Qt = shunt; surf. = surfactant

SUMMARY AND CONCLUSIONS

Although the pathophysiological mechanisms in ARDS and neonatal RDS differ considerably, the pulmonary surfactant system is involved in both syndromes. As extensively outlined in *Chapter 1*, neonatal RDS is characterized by immaturity of the lung, resulting in relative or absolute surfactant deficiency. Due to high surface tensions at the air-liquid interface of the alveolar walls, together with damage caused by mechanical ventilation, high-permeability edema fluid accumulates in the alveolar spaces. ARDS can be caused by different etiologies, such as sepsis, pneumonia, aspiration of gastic contents, and many others (see *Chapter 2*). Due to activation of all kinds of humoral and cellular mechanisms interacting with each other, damage to the alveolar-capillary membranes results, leading to intra-alveolar accumulation of plasma-derived proteins and specific mediators. From experimental studies it is known that plasma proteins inhibit the pulmonary surfactant system in a dose-dependent way. Exogenous surfactant is currently accepted as a valuable treatment for neonatal RDS; also, patients suffering from ARDS have been treated with surfactant. In both neonatal RDS and ARDS it is discussed that in patients not responding to a single dose of exogenous surfactant (so-called "non-responders"), the exogenous surfactant may be inhibited by proteins present in the alveolar space.

In *Chapter 3* it was demonstrated that intratracheal instillation of both pulmonary edema fluid and homologous plasma influenced lung function in healthy rats. It could also be shown that exogenous surfactant, which normally restores gas exchange in surfactant-depleted lungs, failed to do so when mixed with either edema fluid or plasma; these mixtures showed high minimal surface tensions compared to exogenous surfactant alone. In contrast to rats receiving plasma, bronchoalveolar lavage fluid of rats receiving edema fluid showed a high percentage of neutrophils, suggesting the presence of chemotactic factors in the edema fluid. It was concluded that both edema fluid and plasma have the same inhibitory capacity on surfactant function.

To investigate the hypothesis that the inhibitory effect of plasma proteins on (exogenous) surfactant may be dose-dependent, we designed the study in *Chapter 4* in which an exogenous surfactant preparation was mixed with heterologous (human) plasma. Exogenous surfactant concentrations ranged from 50, 100 to 300 mg/kg body weight;

plasma at 4 ml/kg body weight was mixed with these surfactant preparations. These mixtures were intratracheally administered in rats suffering from respiratory failure after whole-lung lavage. Mixtures with surfactant concentrations of 100 and 300 mg/kg were capable of restoring gas exchange to normal. However, at 100 mg/kg lung function deteriorated, while at 300 mg/kg gas exchange was stabilized during a 3-hour observation period. Also, this model proved to be practical for investigating the influence of plasma proteins on different exogenous surfactant preparations.

In respiratory failure caused by aspiration of gastric contents the surfactant system is involved, probably due to inhibition of surfactant function due to plasma proteins and specific mediators. In *Chapter 5* a model was presented in which rats received hydrochloric acid (HCl), intratracheally. In these animals lung function deteriorated rapidly. Bronchoalveolar lavage (BAL) fluid contained high concentrations of proteins, and had high surface tensions. Since these findings suggested inhibition of surfactant function, a study was designed in which the influence of intratracheal instillation of exogenous surfactant at different time points post-HCl instillation on lung function was investigated. It was demonstrated that exogenous surfactant given before development of respiratory failure (i.e. within 10 min post-HCl instillation) could prevent deterioration of lung function. After development of respiratory failure, exogenous surfactant served only to prevent further deterioration of lung function. In clinical practice, aspiration of gastric contents is often detected after development of respiratory failure. For this reason, it would not always be possible to give exogenous surfactant as prophylaxis after aspiration of gastric contents. Therefore, a study was designed to investigate how to treat respiratory failure due to HCl aspiration with exogenous surfactant (*Chapter 6*). It was demonstrated that blood gases improved after protein-rich edema fluid was removed by means of BAL, directly followed by exogenous surfactant administration. Another way to improve lung function was to lavage lungs with a diluted surfactant suspension, thereby probably removing proteins from the alveolar space and simultaneously treating with exogenous surfactant. This lavage procedure could be repeated, resulting in progressive removal of proteins from the alveolar space and improvement of lung function.

In the acute phase of pneumonia, protein-rich edema fluid accumulates in the alveolar space. In the past we demonstrated that exogenous surfactant therapy could

restore lung function in rats and mice suffering from pneumonia due to Sendai virus and influenza A virus infection, respectively. In *Chapter 7* we demonstrated that exogenous surfactant therapy could improve oxygenation in rats suffering from pneumonia caused by pneumocystis carinii infection. Although it is difficult to extrapolate to the clinical situation, exogenous surfactant therapy could be of value in patients suffering from acute respiratory failure due to, or accompanied by, pneumonia. Also, it is hypothesized that exogenous surfactant could serve as a carrier for antibiotics, this way simultaneously restoring gas exchange and delivering drugs to the site of infection.

Natural exogenous surfactant preparations used in clincal studies contain proteins foreign to the patient. As the alveolar-capillary membrane in patients receiving exogenous surfactant is highly permeable to macromolecules, it is likely that some of the exogenous surfactant is lost into the circulation. Therefore, it is reasonable to assume that antibodies may be developed against the surfactant-associated proteins. In the study presented in *Chapter 8* exogenous surfactant was mixed with a monoclonal antibody directed to a 11 kDa surfactant-associated protein. This mixture was not able to restore gas exchange in rats suffering from respiratory failure after whole-lung lavage. Although surfactant-antisurfactant immune complexes have been detected in neonates with RDS, no effect on exogenous surfactant therapy has been reported. These findings also demonstrate the importance of the 11 kDa (probably SP-B) for normal surfactant function.

The work presented in this thesis demonstrates that exogenous surfactant therapy can restore lung function in different animal models of acute respiratory failure. In rats suffering from respiratory failure due to pneumocystis carinii pneumonia a single dose of exogenous surfactant was sufficient to restore lung function, whereas after deterioration of lung function after HCl aspiration, exogenous surfactant was only capable to restore gas exchange after edema fluid was removed by BAL, or BAL with a diluted surfactant suspension. It is concluded that exogenous surfactant therapy can only succeed when given in sufficiently high concentrations: there should be a favorable surfactant-inhibitor ratio. For clinical practice this means that either exogenous surfactant has to be given until gas exchange improves ("titrate"), or that proteins have to be removed from the alveolar space by means of BAL. The way to perform this latter possibility has yet to be investigated.

SAMENVATTING EN CONCLUSIES

Hoewel de pathofysiologische mechanismen in ARDS (adult respiratory distress syndrome) en neonatale RDS (respiratory distress syndrome) verschillen, is in beide syndromen het long surfactant systeem betrokken. Zoals uitgebreid ter sprake kwam in *Hoofdstuk 1*, wordt neonatale RDS gekarakteriseerd door immaturiteit van de long, hetgeen resulteert in een relatief of absoluut tekort aan surfactant. Als gevolg van de hoge oppervlakte spanning aan de lucht-vloeistof overgang en de schade veroorzaakt door kunstmatige beademing, ontstaat er eiwitrijk oedeem in de alveoli. ARDS kan worden veroorzaakt door verschillende ziektebeelden, zoals sepsis, pneumonie, aspiratie van maaginhoud en vele anderen (zie *Hoofdstuk 2*). Activatie van allerlei humorale en cellulaire mechanismen, welke met elkaar interfereren, leidt tot beschadiging van de alveolo-capillaire membraan. Dit laatst leidt tot intraalveolaire ophoping van plasma eiwitten en specifieke mediatoren. Het is bekend dat deze plasma eiwitten het surfactant systeem op een dosis-afhankelijke wijze inhiberen. Op dit moment is exogeen surfactant substitutie geaccepteerd als een waardevolle behandeling voor neonataal RDS; tevens is er een aantal ARDS-patiënten behandeld met surfactant. Bij zowel neonataal RDS als ARDS zijn er patiënten die niet reageren op een enkele dosis exogeen surfactant (zogenaamde "non-responders"). In deze patiënten is het exogeen surfactant waarschijnlijk geïnhibeerd door onder andere plasma eiwitten, welke zich in de alveoli bevinden.

In *Hoofdstuk 3* werd gedemonstreerd dat intratracheale toediening van zowel long oedeem vloeistof als homoloog plasma de longfunctie van gezonde ratten beïnvloedt. Het kon ook worden aangetoond dat exogeen surfactant, welk normaal gesproken de gasuitwisseling herstelt in surfactant-deficiënte longen, hiertoe niet in staat was wanneer het gemengd was met oedeem of plasma. Surfactant gemengd met oedeem of plasma had hoge oppervlakte spanningen vergeleken met surfactant alleen. In tegenstelling tot ratten die plasma hadden gekregen, bevatte bronchoalveolaire lavage (BAL) vloeistof van ratten die oedeem hadden gekregen een hoog percentage neutrofiele granulocyten. Deze laatste bevinding suggereerde de aanwezigheid van chemotactische factoren in oedeem vloeistof. Er werd geconcludeerd dat zowel oedeem als plasma surfactant gelijkwaardig inhiberen.

Om de hypothese te testen dat het inhibitoir vermogen van plasma eiwitten op (exogeen) surfactant dosis-afhankelijk is, werd de volgende studie uitgevoerd (*Hoofdstuk 4*). Exogeen surfactant werd gemengd met heteroloog (humaan) plasma. De concentraties exogeen surfactant varieerden van 50, 100 tot 300 mg/kg lichaamsgewicht. Plasma (4 ml/kg lichaamsgewicht) werd gemengd met exogeen surfactant in genoemde concentraties. De mengsels met surfactant concentraties van 100 en 300 mg/kg waren in staat de gasuitwisseling volledig te herstellen. Echter, bij 100 mg/kg verslechterde de longfunctie zich na verloop van tijd, terwijl bij 300 mg/kg de gasuitwisseling zich gedurende een observatie-periode van 3 uur stabiliseerde. Tevens bleek dit een praktisch model om de invloed van plasma eiwitten op verschillende exogene surfactant preparaten te testen.

Bij respiratoir falen als gevolg van aspiratie van maaginhoud is het surfactant systeem betrokken, waarschijnlijk door inhibitie van de surfactant functie door plasma eiwitten en specifieke mediatoren. In *Hoofdstuk 5* werd een model geïntroduceerd waarbij ratten intratracheaal zoutzuur (HCl) toegediend kregen. Hierdoor verslechterde de longfunctie zich. BAL vloeistof van deze ratten bevatte hoge eiwitconcentraties en had hoge oppervlakte spanningen. Daar deze bevindingen de inhibitie van surfactant suggereerden, werd de volgende studie ontworpen. De invloed van intratracheale toediening van exogeen surfactant op verschillende tijdstippen na HCl aspiratie werd onderzocht. Aangetoond werd dat wanneer exogeen surfactant vóór verslechtering van de longfunctie werd gegeven (d.w.z. binnen 10 minuten na HCl aspiratie), deze verslechtering voorkómen kon worden. Als exogeen surfactant gegeven werd ná verslechtering van de longfunctie verbeterde de gasuitwisseling zich niet. In de praktijk wordt aspiratie echter meestal opgemerkt nadat er al een verslechtering van de longfunctie is opgetreden. Hierdoor zal het niet altijd mogelijk zijn om exogeen surfactant profylactisch na aspiratie van maaginhoud te geven. Om deze reden werd een studie opgezet om een optimale manier te vinden ratten met respiratoir falen na HCl aspiratie te behandelen met exogeen surfactant (*Hoofdstuk 6*). Er werd aangetoond dat de gasuitwisseling verbeterde nadat eiwitrijk oedeem verwijderd was d.m.v. BAL, direct gevolgd door exogeen surfactant substitutie. Tevens kon de longfunctie verbeterd worden door de longen te spoelen met een surfactant suspensie, waarschijnlijk door gelijktijdige verwijdering van plasma eiwitten en behandeling met exogeen surfactant. Deze lavage-

procedure kon herhaald worden, hetgeen resulteerde in een progressieve verwijdering van eiwitten uit de longen en verbetering van de longfunctie.

In de acute fase van pneumonie hoopt eiwitrijk oedeem zich op in de alveoli. In het verleden hebben we aangetoond dat exogeen surfactant substitutie de longfunctie kon verbeteren bij ratten en muizen met pneumonie a.g.v. infectie met, respectievelijk, Sendai virus en influenza A virus. In *Hoofdstuk 7* toonden we aan dat exogeen surfactant substitutie de gasuitwisseling kon verbeteren bij ratten met pneumonie a.g.v. pneumocystis carinii infectie. Hoewel het moeilijk te extrapoleren is naar de klinische situatie, zou exogeen surfactant van waarde kunnen zijn in behandeling van patiënten met respiratoir falen als gevolg van, of in combinatie met, een pneumonie. Tevens zou exogeen surfactant kunnen fungeren als drager voor antibiotica; op deze manier zou tegelijkertijd behandeling van respiratoir falen en lokale applicatie van antibiotica plaatsvinden.

Natuurlijke exogene surfactant preparaten, die in klinische studies gebruikt worden, bevatten lichaams-vreemde eiwitten. Gezien de verhoogde permeabiliteit van de alveolo-capillaire membraan voor macromoleculen in zowel ARDS als neonatale RDS, is het waarschijnlijk dat kleine hoeveelheden exogeen surfactant in de bloedsomloop terecht komen. Om deze reden is het aannemelijk dat antilichamen gevormd worden tegen surfactant-eiwitten. In de studie in *Hoofdstuk 8* werd exogeen surfactant gemengd met een monoklonaal antilichaam gericht tegen een 11 kDa surfactant-eiwit. Dit mengsel bleek niet in staat te zijn de longfunctie te herstellen bij ratten met respiratoir falen a.g.v. surfactant-deficiëntie. Hoewel surfactant-antisurfactant immuuncomplexen aangetoond zijn in neonaten met RDS, is er geen invloed beschreven op het resultaat van exogeen surfactant behandeling. De bevindingen van deze studie tonen tevens het belang van het 11 kDa surfactant-eiwit (waarschijnlijk SP-B) voor de functie exogeen surfactant.

De studies in dit proefschrift tonen in verschillende diermodellen voor acuut respiratoir falen aan dat exogeen surfactant de longfunctie kan herstellen. Bij ratten lijdend aan respiratoir falen a.g.v. pneumocystis carinii pneumonie, was een enkele dosis exogeen surfactant in staat om de longfunctie te herstellen. Na verslechtering van de longfunctie a.g.v. HCl aspiratie, kon exogeen surfactant slechts de gasuitwisseling verbeteren nadat eiwitrijk oedeem d.m.v. BAL verwijderd was, óf door BAL met een

surfactant suspensie. De conclusie is dat exogeen surfactant behandeling slechts kan slagen wanneer voldoende hoog gedoseerd wordt: er dient altijd een gunstige surfactant/eiwit ratio te zijn. In de praktijk betekent dit dat óf exogeen surfactant gegeven moet worden tot de gasuitwisseling verbetert ("titreren"), óf dat eiwitten verwijderd moeten worden d.m.v. BAL, gevolgd door exogeen surfactant substitutie. Het zal nog uitgezocht moeten worden op welke manier deze laatste optie kan worden uitgevoerd.

DANKWOORD

Het in dit proefschrift beschreven onderzoek werd binnen de afdeling Anaesthesiologie van de Erasmus Universiteit Rotterdam verricht. Velen binnen en buiten deze afdeling hebben meegewerkt aan de totstandkoming van dit proefschrift. Ik wil een ieder hartelijk danken voor hun inzet en belangstelling. Een aantal mensen hebben een belangrijke rol gespeeld.

In de eerste plaats Karin. Haar wil ik bedanken voor alles. Het is onbegrijpelijk waar ze telkens weer de energie en het geduld vandaan haalt om mijn gemopper aan te horen.

Mijn ouders. Hun nimmer afnemende vertrouwen in mij en hun belangstelling voor mijn werk waren en zijn essentieel.

Prof. Dr. B. Lachmann. Hem bedank ik voor de mij geboden kansen. De vele discussies die we hadden hebben altijd weer tot resultaat geleid.

Prof. Dr. W. Erdmann, Prof. Dr. H.A. Bruining, Prof. Dr. C. Hilvering en Prof. Dr. L.M.G. van Golde dank ik voor hun bereidheid dit proefschrift op wetenschappelijke inhoud te beoordelen.

I wish to express my gratitude to Dr. C.J. Morley for his willingness to be a member of the committee during the defence of my thesis.

Jelle Bos. (S)amen uit (s)amen thuis!

Diederik Gommers. Hoe dit vervelende mannetje één van mijn beste vrienden werd blijft mij een raadsel.

Ralph So ("hoezo dan?"). Een patente remedie tegen lastige statistische vragen van Reviewers.

Annemarie ("Pneutje") van 't Veen. Haar bedank ik voor haar vermogen mij telkens weer te verbazen.

Arie Kok. Zonder zijn inzet en vaardigheden was het allemaal nooit zó uit de hand gelopen.

Laraine Visser. Zonder haar was er nooit iets in fatsoenlijk "English" de deur uitgegaan. Haar "farewell kiss" op de dichtgeplakte envelop speelde een cruciale rol in het geaccepteerd krijgen van een artikel.

Verder was er een groot aantal mensen verantwoordelijk voor een goede werksfeer, plezier, geroddel en vriendschap: Robert Jan Houmes, Ahmet Tütüncü, Rob ("Bobo")

Tenbrinck, Geert-Jan van Daal ("een gewaardeerd collega"), Arthur Voogd, Edwin Hendrik, N. Govindarajan, Ron van der Kamp, Erwin de Buijzer, Marieke Vergeer en Elke Scheffers.

I thank Prof. Dr. D.S. Strayer, Prof. Dr. T.A. Merritt and Prof. Dr. E. Hannappel for their valuable contribution to some of the work presented in this thesis.

Ten slotte bedank ik Johan Mouton, Dr. J.F. Sluiters en Ad Luijendijk van de afdeling Klinische Microbiologie en Moniek de Maat ven de afdeling Inwendige Geneeskunde II voor hun essentiële bijdrage aan dit proefschrift.

CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren op 2 juni 1965 te Hilversum. In 1984 behaalde hij het VWO-diploma aan de scholengemeenschap "J.C. de Glopper" te Capelle aan den IJssel. Aansluitend begon hij in 1984 met de studie Geneeskunde aan de Erasmus Universiteit te Rotterdam, alwaar hij in 1989 het doctoraal examen behaalde. Gedurende zijn studie vervulde hij een student-assistentschap bij Prof. Dr. B. Lachmann op de afdeling Experimentele Anaesthesiologie van de Erasmus Universiteit. Na zijn doctoraal examen trad hij in dienst bij de Erasmus Universiteit als wetenschappelijk onderzoeker alwaar hij onderzoek deed naar long surfactant onder begeleiding van Prof. Dr. B. Lachmann. In 1991 begon hij zijn co-assistentschappen, welke in maart 1993 afgerond zullen worden. Tijdens deze co-assistentschappen werkte hij aan de totstandkoming van dit proefschrift.

LIST OF PUBLICATIONS

1. E.P. Eijking, J.A.H. Bos, S. Armbruster, W. Schelter, W. Gumbrecht, W. Erdmann, B. Lachmann. Online measurements of SaO_2, Ht and Hb using a micro transmission cell. Adv Exp Med Biol 1990; 277: 53-57
2. J.A.H. Bos, W. Schelter, W. Gumbrecht, B. Montag, E.P. Eijking, S. Armbruster, W. Erdmann, B. Lachmann. Development of a micro transmission cell for in vivo measurement of SaO_2 and Hb. Adv Exp Med Biol 1990; 277: 47-52
3. W. Schelter, W. Gumbrecht, B. Montag, J. Bos, E. Eijking, B. Lachmann. A micro transmission cell for monitoring of oxygen saturation and hemoglobin concentration. Sensors and Actuators 1990; B1: 495-498
4. W. Gumbrecht, W. Schelter, B. Montag, J.A.H. Bos, E.P. Eijking, B. Lachmann. Monitoring of blood PO_2 with a thin film amperometric sensor. IEEE, Transducers 1991, Digest of technical papers, 85-87
5. E.P. Eijking, D.S. Strayer, G.J. van Daal, B. Lachmann. Effects of antisurfactant antibodies on the course of mild respiratory distress syndrome. Pathobiology 1991; 59: 96-101
6. E.P. Eijking, D.S. Strayer, G.J. van Daal, R. Tenbrinck, T.A. Merritt, E. Hannappel, B. Lachmann. In vivo and in vitro inactivation of bovine surfactant by an anti-surfactant monoclonal antibody. Eur Respir J 1991; 4: 1245-1250
7. G.J. van Daal, K.L. So, D. Gommers, E.P. Eijking, R.B. Fiévez, M.J. Sprenger, D.W. van Dam, B. Lachmann. Intratracheal surfactant administration restores gas exchange in experimental adult respiratory distress syndrome associated with viral pneumonia. Anesth Analg 1991; 72: 589-595
8. E.P. Eijking, G.J. van Daal, R. Tenbrinck, A. Luijendijk, J.F. Sluiters, E. Hannappel, B. Lachmann. Effect of surfactant replacement on pneumocystis carinii pneumonia in rats. Intensive Care Med 1991; 17: 475-478
9. G.J. van Daal, J.A.H. Bos, E.P. Eijking, D. Gommers, E. Hannappel, B. Lachmann. Surfactant replacement therapy improves pulmonary mechanics in end-stage influenca A pneumonia in mice. Am Rev Respir Dis 1992; 145: 859-863
10. B. Lachmann, E.P. Eijking. Pulmonary surfactant: basic physiology and its use for replacement therapy. In: Drug targeting and delivery. Concepts in dosage form

design. H.E. Junginger (ed.). Ellis Horwood Limited. New York, 1992, 129-154

11. E.P. Eijking, J.A.H. Bos, W. Schelter, W. Gumbrecht, W. Erdmann, B. Lachmann. A new catheter for quasi-continuous measurement of arterial partial oxygen pressure. Adv Exp Med Biol 1992; 316: 71-74
12. G.J. van Daal, E.P. Eijking, K.L. So, R.B.A.M. Fiévez, M.J.W. Sprenger, D.W. van Dam, W. Erdmann, B. Lachmann. Acute respiratory failure during pneumonia induced by Sendai virus. Adv Exp Med Biol 1992; 316: 319-326
13. E.P. Eijking, D. Gommers, J. Kullander, E. de Buijzer, R.W. Beukenholdt, B. Lachmann. Different surfactant treatment strategies for respiratory failure induced by hydrochloric acid aspiration in rats. Adv Exp Med Biol 1992; 317: 349-355
14. E.P. Eijking, D. Gommers, K.L. So, M.P.M. de Maat, J.W. Mouton, B. Lachmann. Prevention of respiratory failure after hydrochloric acid aspiration by intratracheal surfactant instillation in rats. Anesth Analg 1993; 76: 472-477
15. E.P. Eijking, D. Gommers, K.L. So, M. Vergeer, B. Lachmann. Surfactant treatment of respiratory failure induced by hydrochloric acid aspiration in rats. Anesthesiology 1993 (in press)
16. J.A.H. Bos, P. Wollmer, E.P. Eijking, D. Gommers, W. van Gelder, W.H. Bakker, B. Lachmann. Pulmonary technetium ^{99m}Tc-human serum albumin clearance and effects of surfactant replacement after lung lavage in rabbits. Clin Physiol (submitted for publication)
17. E.P. Eijking, D. Gommers, K.L. So, R. van der Kamp, M.P.M. de Maat, B. Lachmann. In vivo and in vitro inhibition of surfactant function by protein-rich pulmonary edema fluid. Anesth Analg (submitted for publication)
18. B. Lachmann, E.P. Eijking, K.L. So, D. Gommers. In vivo evaluation of the inhibitory capacity of human plasma on exogenous surfactant function. Intensive Care Med (submitted for publication)

ABSTRACTS

1. E.P. Eijking, J.A.H. Bos, W. Schelter, W. Gumbrecht, S. Armbruster, B. Lachmann. Quasikontinuierliche Messungen von SaO_2, Hb, und Ht mit einer miniaturisierten Spektrophotometriezelle. Anaesthesist 1989; 38 (Suppl 1): 435

2. E.P. Eijking, G.J. van Daal, R. Tenbrinck, J.F. Sluiters, B. Lachmann. Surfactant replacement in rats with pneumocystis carinii pneumonia (PCP). Intensive Care Med 1990; 16 (Suppl 1): S87
3. E.P. Eijking, D. Gommers, E. Hannappel, B. Lachmann. Prevention of respiratory failure after HCl aspiration by intratracheal surfactant instillation in rats. Anaesthesist 1991; 40 (Suppl 2): S203
4. K.L. So, E.P. Eijking, D. Gommers, E. de Buijzer, R.W. Beukenholdt, B. Lachmann. Different surfactant treatment strategies for respiratory failure induced by hydrochloric acid aspiration in rats. Intensive Care Med 1992; 18 (Suppl 2): S111