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Dose-response comparisons of five lung surfactant factor (LSF) preparations in an animal model of adult respiratory distress syndrome (ARDS)

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1 We have examined the effects of five different lung surfactant factor (LSF) preparations in the rat lung lavage model. In this model repetitive lung lavage leads to lung injury with some similarities to adult respiratory distress syndrome with poor gas exchange and protein leakage into the alveolar spaces. These pathological sequelae can be reversed by LSF instillation soon after lavage.

2 The tested LSF preparations were: two bovine: Survanta and Alveofact: two synthetic: Exosurf and a protein-free phospholipid based LSF (PL-LSF) and one Recombinant LSF at doses of 25, 50 and 100 mg kg^{-1} body weight and an untreated control group.

3 Tracheotomized rats (10-12 per dose) were pressure-controlled ventilated (Siemens Servo Ventilator 900C) with 100% oxygen at a respiratory rate of 30 breaths min^{-1}, inspiration expiration ratio of 1 : 2, peak inspiratory pressure (PIP) of 28 cmH_2O at positive end-expiratory pressure (PEEP) of 8 cmH_2O. Two hours after LSF administration, PEEP and in parallel PIP was reduced from 8 to 6 (1st reduction), from 6 to 3 (2nd reduction) and from 3 to 0 cmH_2O (3rd reduction).

4 Partial arterial oxygen pressure (Pa_{O_2}, mmHg) at 5 min and 120 min after LSF administration and during the 2nd PEEP reduction (Pa_{O_2}(PEEP23/3)) were used for statistical comparison. All LSF preparations caused a dose-dependent increase for the Pa_{O_2}(120'), whereas during the 2nd PEEP reduction only bovine and recombinant LSF exhibited dose-dependency. Exosurf did not increase Pa_{O_2} after administration of the highest dose. At the highest dose Exosurf exerted no further improvement but rather a tendency to relapse. The bovine and the Recombinant LSF are superior to both synthetic LSF preparations.

5 In this animal model and under the described specific ventilatory settings, even between bovine LSF preparations there are detectable differences that are pronounced when compared to synthetic LSF without any surfactant proteins. We conclude that the difference between bovine and synthetic LSF preparations can be overcome by addition of the surfactant protein C.

Keywords: Adult respiratory distress syndrome (ARDS)-model; dose-response comparisons; synthetic lung surfactant factor; bovine lung surfactant factor; recombinant lung surfactant factor; gas exchange; surfactant protein C (SP-C)

Introduction

Since its first description by Ashbaugh *et al.* (1967) mortality due to the adult respiratory distress syndrome (ARDS), currently about 50-65% (Shale, 1987), has not changed (Shale, 1987; Villar & Slutsky 1989), suggesting little advance in therapy. Despite pharmacological approaches such as therapy with prostaglandin E_1 (PGE_1) (Russel *et al.*, 1990) corticosteroids (Bernard *et al.*, 1987), conventional ventilatory interventions (Marini & Kelsen, 1992; Swami & Keogh, 1992) or even extracorporeal membrane oxygenation (ECMO) (Evans & Keogh, 1991) no therapy has so far demonstrated improved survival of patients in clinical testing, whereas optimal ventilator settings have shown some beneficial effects related to outcome (Hickling *et al.*, 1990; East *et al.*, 1992). A recently published report concerning the use of ECMO also shows controversial beneficial effects of ECMO (Lewandowski *et al.*, 1992), but the techniques used are very complex, labour intensive and limited to a few clinical centres (Swami & Keogh, 1992).

For the development of alternate therapeutic interventions a major focus of current research is the role of the lung surfactant factor (LSF) system. Alterations in the surfactant system are proposed to contribute significantly to the pathophysiology of human ARDS (Lachmann & Danzmann, 1984). Changes in LSF composition (Lachmann & Danzmann, 1984) and function (Lachmann & Danzmann, 1984; Gregory *et al.*, 1991) are detectable in the lavage fluid of ARDS patients. Moreover, there are a few case reports on surfactant treatment in adult subjects (Lachmann, 1987; Richman *et al.*, 1989; Nosaka *et al.*, 1990) which reported some beneficial effects in ARDS patients (for review see Gommers & Lachmann, 1993).

As several animal models of ARDS are now available, it is reasonable to test the efficacy of LSF preparations in suitable and well-defined animal models, before testing such preparations in man. Furthermore, it is thus possible to compare the efficacy of different LSF preparations under systematic and standardized conditions in dose-response experiments. In various animal species, including rats, surfactant depletion by repetitive total lung lavage leads to lung injury with some similarities to that seen in ARDS (Lachmann *et al.*, 1980; van Daal *et al.*, 1991). The pathological changes observed are atelectasis, protein leakage leading to formation of hyaline membranes and oedema (Lachmann *et al.*, 1980; Berggren *et al.*, 1986). These changes lead to severe deterioration of gas exchange (Berggren *et al.*, 1986). Several reports consistently showed alleviation of these pathological sequelae after administration of LSF (Lachmann *et al.*, 1980; Berggren *et al.*, 1986; Lachmann *et al.*, 1994). We have employed a modification of the lung lavage model originally described by Lachmann *et al.* (1980). The aim of our study was to assess dose-response curves of five different LSF preparations in the rat lung lavage model under conditions standardized with respect

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to the ventilatory settings as well as the mode and volume of LSF administration. The examined LSF preparations were: (1) a bovine LSF preparation, Alveofact (Dr Karl Thomae GmbH, Biberach, Germany), (2) Survanta (Abbott GmbH, Wiesbaden, Germany), another bovine LSF preparation, (3) a synthetic LSF, Exosurf (Burroughs Wellcome Co., Research Triangle Park, U.S.A.) that contains only one phospholipid and no surfactant proteins, (4) another synthetic LSF, (PL-LSF, Byk Gulden, Konstanz, Germany) that contains two phospholipids and no surfactant proteins and (5) a Recombinant LSF (Byk Gulden, Konstanz, Germany). We selected these LSF preparations in order to compare the efficacy of the two natural, bovine LSF preparations and the two protein-free synthetic LSF preparations with a Recombinant LSF preparation that contains human identical s,s-dipalmitoylated surfactant protein C as potential treatments of ARDS patients.

Preliminary results were presented at the ALA/ATS meeting in Miami (Häfner *et al.*, 1993).

Methods

Preparation of the rats

This study protocol was reviewed and approved by the Laboratory Animal Care Committee at the district presidency of Freiburg, Germany. The study was performed with a total of 186 male Sprague Dawley rats (Harlan CBP, Zeist, The Netherlands), weighing 230–290 g.

After induction of anaesthesia with a halothane nitrous oxide (N_2O), oxygen (O_2) mixture (1–2% halothane, 70% N_2O and 28–29% O_2) a catheter was placed in one carotid artery. These catheters contained heparinized, isotonic saline solution (from the stock solution [5000 iu heparin-Na ml^{-1}], 0.5 ml was given in 250 ml 0.9% NaCl solution). Before tracheotomy the animals received an intraperitoneal (i.p.) injection of pentobarbitone (stock solution: 60 mg ml^{-1}; 1 ml kg^{-1} body weight). The trachea of each animal was cannulated with a tracheal tube (internal diameter: 1.8 mm; external diameter: 2.4 mm). The tube was secured by ligature. Before artificial ventilation was started, the animals were given an i.m. injection of pancuronium bromide as muscle-relaxant (2 mg kg^{-1}). During the experiment additional pentobarbitone (0.25 ml kg^{-1} of the stock solution) was given i.p. at hourly intervals. Pancuronium bromide was additionally injected i.m. if spontaneous breathing was observed. Six animals were connected to a distributor unit that in turn was connected to a Servo Ventilator. The animals were ventilated simultaneously at a respiratory rate of 30 breaths min^{-1}, a fraction of inspired oxygen (FiO_2) of 1.0, an inspiration expiration ratio of 1:2 and a peak inspiratory pressure (PIP) of 15 cmH_2O which included a positive end-expiratory pressure (PEEP) of 2 cmH_2O.

Protocols for the animal experiments

The reported parameters are arterial PaO_2 and $PaCO_2$. At the start of the experiment, blood was taken from the arterial catheter to determine pretreatment values of the animals under the described ventilatory settings. Only animals with PaO_2 values of more than 480 mmHg were included in the experiments. Before lavage the peak inspiration pressure (PIP) was raised to 28 cmH_2O and PEEP to 8 cmH_2O. The animals were then subjected to multiple lavage (6–8 times) with 1 ml 30 g^{-1} body wt. of isotonic saline solution warmed to body temperature. To avoid metabolic acidosis, 4 ml kg^{-1} of a glucose/$NaHCO_3$-solution (5 g glucose-monohydrate and 8.4 g $NaHCO_3$ dissolved in 100 ml 0.9% NaCl-solution) were given by i.p. injection to each animal after lavage. Additionally, the same amount of glucose/$NaHCO_3$ was given if the HCO_3-value in the arterial blood gas analysis fell below 20 mmol l^{-1} during the experiment. Five minutes after the PaO_2 fell to between 50 and 110 mmHg, LSF was instilled intratracheally as described below. Blood gases were subsequently determined at 5, 30, 60, 90 and 120 min after LSF instillation. After this, PIP was lowered from 28 cmH_2O to 26 cmH_2O and PEEP from 8 to 6 cmH_2O (= 1st PEEP reduction). This was followed by a 2nd PEEP reduction from 6 to 3 cmH_2O and a 3rd PEEP reduction to 0 cmH_2O PEEP. Five minutes after each PEEP reduction, blood gases were measured (for the experimental scheme see Figure 1). Immediately after the measurement of the blood gases of the last animal, PEEP was lowered as described.

Instruments

Blood gas analysis was performed with a blood gas analyzer (Radiometer Copenhagen ABL 500, Radiometer Deutschland GmbH, Willich, Germany). Ventilation of the animals was performed with a Servo Ventilator (900C, SIEMENS-ELEMA, Solna, Sweden). For induction of anaesthesia a halothane vaporizer (Draegerwerk GmbH, Lübeck, Germany) was used.

Surfactants

The following were used: Alveofact (Batch No. 102108, Dr Karl Thomae GmbH, Biberach, Germany) is a natural surfactant purified from bovine lung lavage containing varying amounts of surfactant proteins B and C. It is available as a ready-to-use suspension. Each vial contains 1.2 ml solution containing 50 mg phospholipids (PL). Survanta (Batch No. 56-763 AN-21, Abbott GmbH, Wiesbaden, Germany) is also a natural, bovine surfactant. It is obtained from minced cow lungs containing varying amounts of surfactant proteins B and C and is enriched with dipalmitolphosphatidylcholine, palmitic acid and tripalmitin. It is also available as a ready-to-use suspension. Each vial contains 8 ml solution at a concentration of 25 mg PL per ml. Exosurf (Batch No. PN17191, Burroughs Wellcome Co., Research Triangle Park, U.S.A.) is a protein-free synthetic surfactant made of dipalmitoylphosphatidylcholine (DPPC), tyloxapol and cetyl alcohol. It is a lyophilisate that needs to be resuspended with a supplied solvent to achieve a volume of 8 ml/vial. For the purpose of this study each vial was resuspended with 4.3 ml to achieve a concentration of 25 mg DPPC per ml. Another protein-free phospholipid-based surfactant (PL-LSF) made of diplamitoylphophatidylcholine (DPPC) and palmitoyloleoylphosphatidylglycerol (POPG) at a ratio of 70 : 30 plus 2.5% (w/w) palmitic acid as related to phospholipids. The PL-LSF was prepared as a lyophilisate that had to be resuspended before use. Therefore, each vial of PL-LSF was resuspended with 6 ml 0.9% NaCl solution giving a concentration of 25 mg PL per ml. A Recombinant LSF (Byk Gulden, Konstanz, Germany) made of phospholipids (dipalmitoylphosphatidylcholine and palmitoyloleoylphosphatidylglycerol at a ratio of 70 : 30) plus 2.5% (w/w) palmitic acid and 2% human identical s,s-dipalmitoylated surfactant protein C as related to phospholipids. The Recombinant LSF was also prepared as a lyophilisate that had to be resuspended before use. Therefore, each vial of recombinant LSF was resuspended with 6 ml 0.9% NaCl solution giving a concentration of 25 mg PL per ml.

Dosage

The different LSF preparations were instilled intratracheally (i.t.) at doses of 25, 50 and 100 mg total phospholipids per kg body weight in a volume of 1.2 ml per animal. To achieve the required concentrations of 6.25, 12.5 and 25 mg total phospholipids per 1.2 ml the LSF preparations were diluted with 0.9% saline solution.

Mode of LSF administration: Administration was performed using 5 ml syringes which contained 1.2 ml LSF solution and 3.8 ml air. The syringes were connected to the tracheal tubes of the animals and LSF was administered first, followed directly by the administration of air. The syringe was disconnected and then a further 3 ml of air was administered into the tracheal

tube of each animal to empty both, the syringe and the tube.

In the control group the animals underwent multiple lavage only, without any administration of surfactant.

Statistics

For each LSF preparation and each dose level and also for the control group 10–12 rats were used. The influence of LSF instillation on the test parameters (Pa_{O_2} and Pa_{CO_2}) was described in absolute values showing mean and standard deviation (s.d.). Values for the Pa_{O_2} at 5 and 120 min after LSF administration as well as for the different PEEP reductions are presented. From the values of Pa_{O_2} during the 2nd PEEP reduction (Pa_{O_2}(PEEP23/3)) dose-response curves were plotted. These values were also used to test for statistically significant differences between the LSF preparations at each dose level. Additionally, each dose was compared to the control group.

Before testing for inter-group differences we performed the Dixon test to eliminate outliers. Homogeneity of variances was tested with Bartlett's test and as the variances differed significantly we used the paired Student-Welch test with α-adjustment for multiple comparison (Sachs, 1986). The comparison between the different LSF preparations and each dose level was performed using the Pa_{O_2}(PEEP23/3).

Results

Feasibility of the comparison

The experimental procedures were validated in pilot experiments prior to the study. The whole study was performed within three months without any dropouts. After lavage none of the animals in the control group showed a spontaneous improvement of the Pa_{O_2} values or the Pa_{CO_2} values (Figure 1).

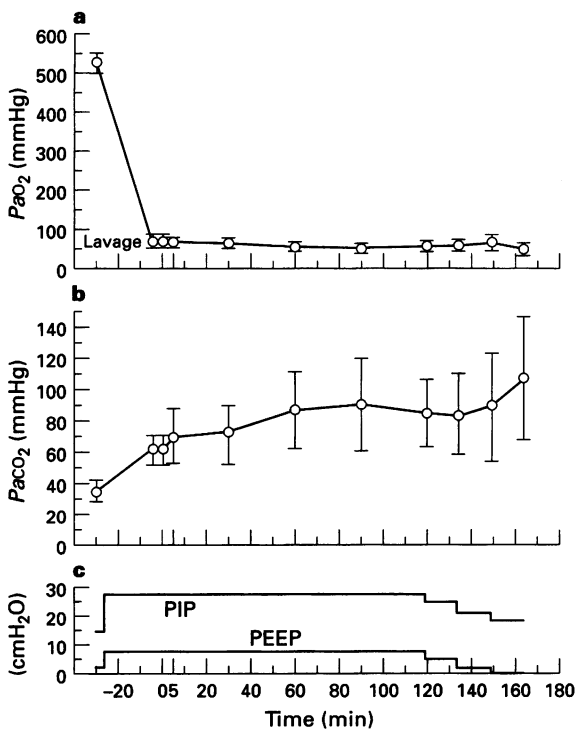

Figure 1 Mean (○) and individual values of Pa_{O_2} (mmHg) (a) and Pa_{CO_2} (mmHg) (b) after ventilation only of lung lavaged rats ($n = 12$). Lavage marks the period where the repetitive lavage was performed. (c) Shows the corresponding time course of the peak inspiratory pressure (PIP, cmH_2O) and the positive end-expiratory pressure (PEEP, cmH_2O) during the experiment.

Time course of the registered parameters

Two hours after administration of the two lowest doses (25 and 50 mg kg^{-1} body wt). of Alveofact Pa_{O_2} decreased compared to the 5 min values of Pa_{O_2}. In contrast, the Pa_{O_2} values of those animals treated with Survanta or Recombinant LSF showed stable values during this period after administration of either doses (Figures 2 and 3). In contrast, animals receiving Exosurf displayed stable values during this period but on a lower level as shown by comparison of the mean curves, but this LSF preparation led to a larger standard deviation during this period. The second synthetic LSF preparation (PL-LSF) showed a decrease of Pa_{O_2} after all three doses during the ventilation period with constant PEEP. The Pa_{O_2} values increased with higher doses but remained below the values of Recombinant LSF and the two bovine LSF preparations. Blood gases after administration of Survanta and Recombinant LSF improved within 5 min after administration and remained stable until 2 h after LSF administration. During this period PEEP remained unchanged. Comparing the different doses it is obvious that with increasing dose the response becomes more homogeneous and the variability between the animals becomes smaller. This dose-dependent effect is less pronounced after administration of Exosurf. Response to this LSF preparation showed a great variability, even at a dose of 100 mg kg^{-1} (Figure 4).

During the PEEP reduction manoeuvre, significant dose-dependent impairments of Pa_{O_2} were observed after administration of the two bovine LSF preparations, Recombinant LSF and the protein-free PL-LSF. The synthetic LSF Exosurf behaved differently: the lowest dose exerted moderate activity whereas after the highest dose a decrease in activity with respect to Pa_{O_2} values were observed. However, in the control group none of the animals showed any improvements in the low Pa_{O_2} level at any time after lavage (Figure 1).

Pa_{CO_2} values showed an almost inverse pattern of response to the Pa_{O_2} values after administration of the different LSF preparations at all three doses (Table 1–3). During lavage Pa_{CO_2} increased and after administration of LSF it decreased. During the PEEP reduction manoeuvre the Pa_{CO_2} increased towards the third PEEP reduction. After administration of Alveofact the changes in Pa_{CO_2} showed a dose-dependency. This was particularly marked when comparing the values during the third PEEP reduction (Tables 1–3). Only the Recombinant LSF behaved in a similar way to Alveofact. Survanta, Exosurf and PL-LSF showed only weak dose-dependency for this parameter. The PL-LSF behaved similarly to Exosurf. The Pa_{CO_2} value at 120 min after LSF administration was only slightly lower than the 5 min value after administration of both protein-free LSF's at all three doses tested. However, in the control group none of the animals reached the Pa_{CO_2} level seen before the start of experiment.

Dose-response curve calculations and statistical inter-group comparison

Dose-response curves (Figure 5) were constructed based on the values of Pa_{O_2} during the 2nd PEEP reduction (Pa_{O_2} (PEEP23/3)).

The results of the comparison of the different LSF preparations at a dose of 25 mg kg^{-1} and in comparison to controls showed that only Recombinant LSF and Survanta were significantly better than controls, whereas Alveofact, Exosurf and PL-LSF did not differ significantly from the control group. In addition, the dose of 25 mg kg^{-1} Alveofact or PL-LSF differed significantly to Survanta ($P \leqslant 0.05$) and to Recombinant LSF ($P \leqslant 0.01$), showing that a dose of 25 mg kg^{-1} Survanta or Recombinant LSF is more effective than a dose of 25 mg kg^{-1} Alveofact or PL-LSF, respectively. There was no significant difference between Exosurf and the other LSF preparations.

Evaluation of the different LSF preparations after administration of 50 mg kg^{-1} LSF showed no significant difference

between the different commercially available LSF preparations and the Recombinant LSF but all surfactants differed to PL-LSF at different levels of significance (Alveofact and Exosurf vs. PL-LSF with $P \leqslant 0.05$ and Recombinant LSF and Survanta vs. PL-LSF with $P \leqslant 0.01$). However, all commercially available LSF preparations and Recombinant LSF were significantly better than controls, but the preparations differed with respect to the significance level (Recombinant LSF and

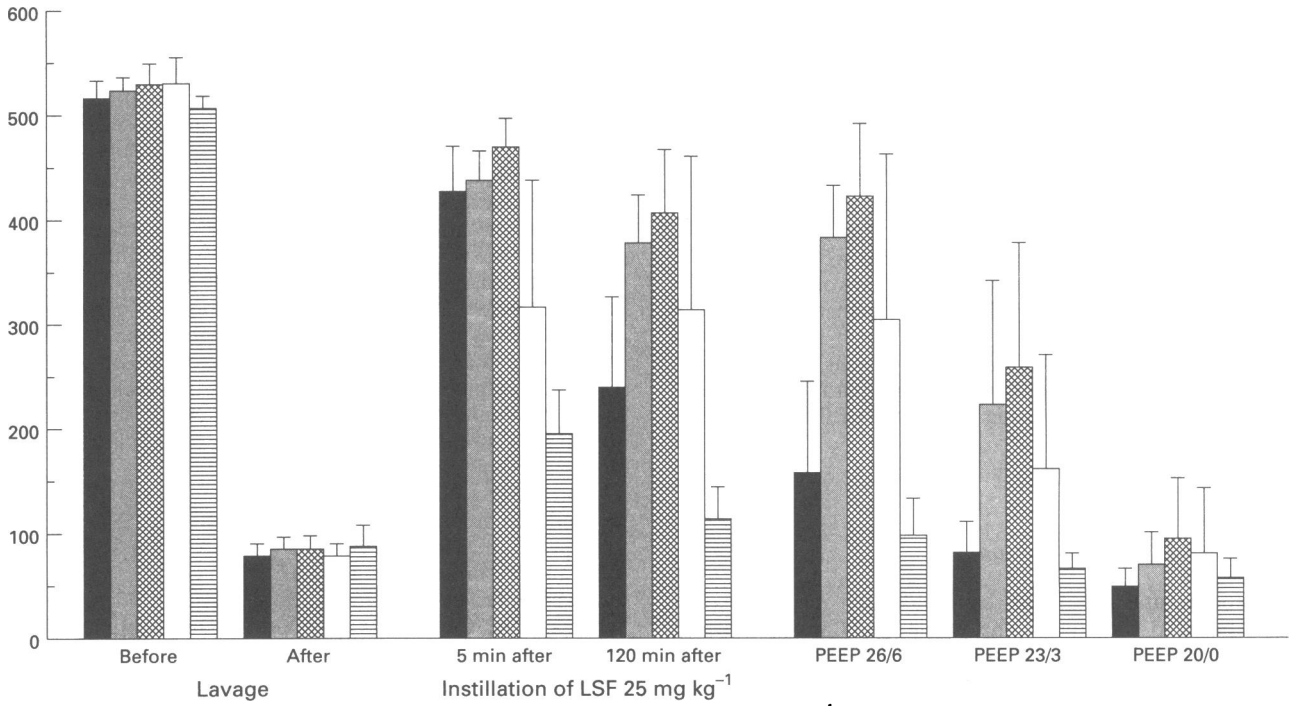

Figure 2 Comparison of Pa_{O_2} (mmHg) values after administration of 25 mg kg^{-1} body weight of Alveofact (solid columns); Survanta (stippled columns); Exosurf (open columns); protein-free, phospholipid-based LSF (hatched columns) and Recombinant LSF (cross-hatched columns). All values are given as mean plus s.d., $n = 10-12$ for each dose. The figure shows the values before and after lavage as well as 5 and 120 min after instillation of 25 mg kg^{-1} body weight of the different LSF preparations. The last three groups of columns show the values during the stepwise decrease of positive end-expiratory pressure (PEEP) to zero.

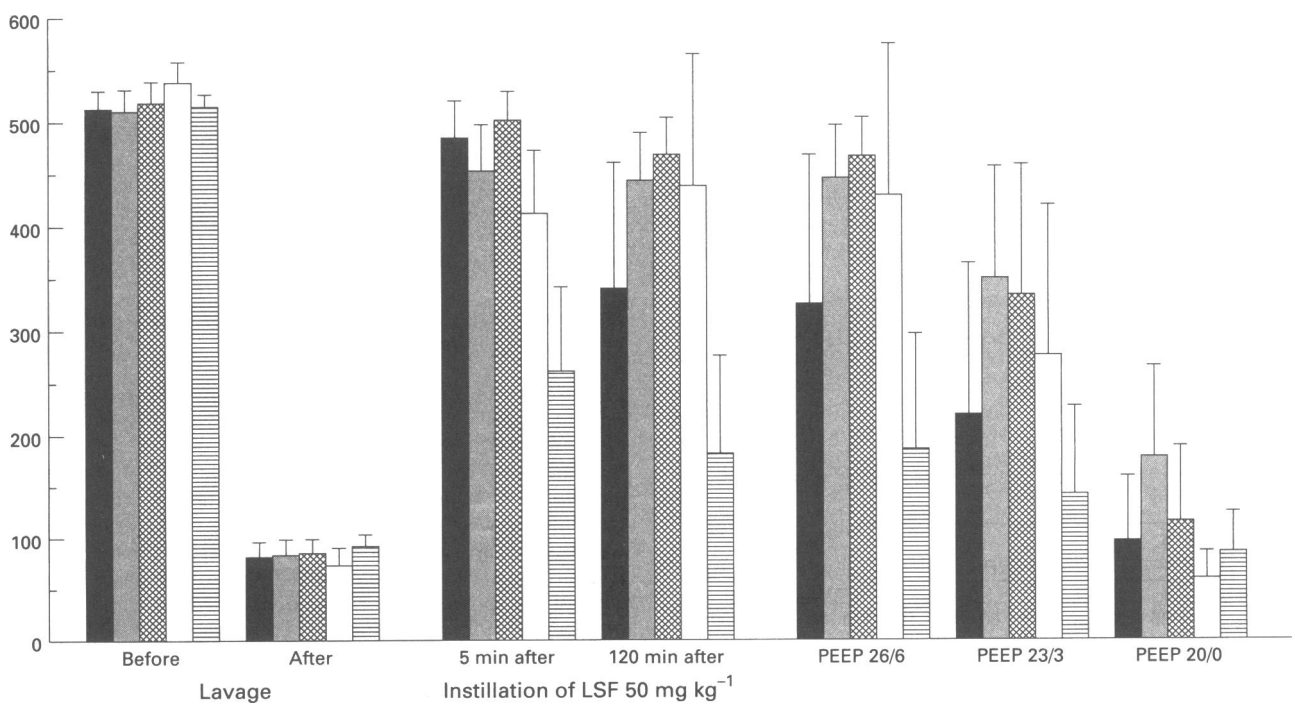

Figure 3 Comparison of Pa_{O_2} (mmHg) values after administration of 50 mg kg^{-1} body weight of Alveofact (solid columns); Survanta (stippled columns); Exosurf (open columns); protein-free, phospholipid-based LSF (hatched columns) and Recombinant LSF (cross-hatched columns). All values are given as mean plus s.d., $n = 10-12$ for each dose. The figure shows the values before and after lavage as well as 5 and 120 min after instillation of 50 mg kg^{-1} body weight of the different LSF preparations. The last three groups of columns show the values during the stepwise decrease of positive end-expiratory pressure (PEEP) to zero.

Survanta $P \leqslant 0.001$, Alveofact and Exosurf $P \leqslant 0.01$). At this dose level there was no statistically significant difference between PL-LSF and controls.

Comparisons based on a dose of 100 mg kg^{-1} of the different LSF preparations showed that Recombinant LSF, Alveofact and Survanta exerted significantly better effects than controls with $P \leqslant 0.001$, whereas this comparison for Exosurf resulted in significance only at the $P \leqslant 0.05$ level and for PL-LSF at the $P \leqslant 0.01$ level. Furthermore, Recombinant LSF, Alveofact and Survanta showed no significant difference when compared with each other, but they differed significantly from Exosurf and PL-LSF. Recombinant LSF and Alveofact dif-

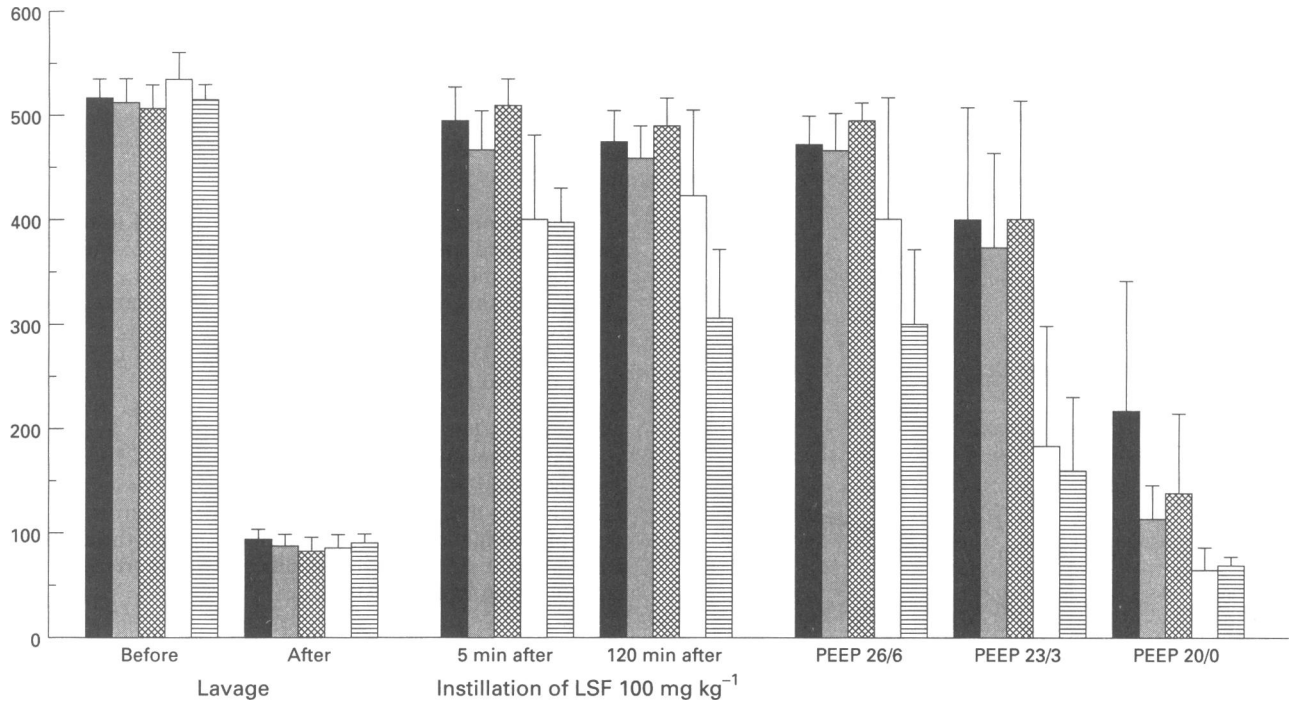

Figure 4 Comparison of Pa_{O_2} (mmHg) values after administration of 100 mg kg^{-1} body weight of Alveofact (solid columns); Survanta (stippled columns); Exosurf (open columns); protein-free, phospholipid-based LSF (hatched columns) and Recombinant LSF (cross-hatched columns). All values are given as mean plus s.d., $n = 10-12$ for each dose. The figure shows the values before and after lavage as well as 5 and 120 min after instillation of 100 mg kg^{-1} body weight of the different LSF preparations. The last three groups of columns show the values during the stepwise decrease of positive end-expiratory pressure (PEEP) to zero.

Table 1 Comparison of $Paco_2$ (mmHg) values after administration of 25 mg kg^{-1} body weight of Alveofact, Survanta, Recombinant LSF, protein-free, phospholipid-based LSF and Exosurf

	Before	*After*	*5 min after*	*120 min after*	*PEEP reductions*		
	Lavage		*instillation of 25 mg kg^{-1} LSF*		*26/6*	*23/3*	*20/0*
Alveofact	39 ± 5	72 ± 13	61 ± 7	62 ± 11	62 ± 14	75 ± 26	109 ± 39
Survanta	39 ± 10	66 ± 13	60 ± 8	47 ± 7	44 ± 5	52 ± 8	86 ± 19
Recombinant LSF	34 ± 7	60 ± 6	49 ± 4	48 ± 5	44 ± 4	48 ± 12	73 ± 27
Protein-free LSF	33 ± 5	65 ± 17	64 ± 17	67 ± 19	69 ± 24	89 ± 20	73 ± 27
Exosurf	35 ± 9	60 ± 12	59 ± 13	51 ± 14	48 ± 14	59 ± 26	89 ± 30

All values are given as mean ± standard deviation, $n = 10$-12 for each dose. The table shows the values before and after lavage as well as 5 and 120 min after instillation of 25 mg kg^{-1} body weight of the different LSF preparations. The last three columns show the values during the stepwise decrease of positive end-expiratory pressure (PEEP) to zero.

Table 2 Comparison of $Paco_2$ (mmHg) values after administration of 50 mg kg^{-1} body weight of Alveofact, Survanta, Recombinant LSF, protein-free, phospholipid-based LSF and Exosurf

	Before	*After*	*5 min after*	*120 min after*	*PEEP reductions*		
	Lavage		*instillation of 50 mg kg^{-1} LSF*		*26/6*	*23/3*	*20/0*
Alveofact	42 ± 7	76 ± 11	60 ± 7	56 ± 11	55 ± 14	57 ± 20	78 ± 33
Survanta	38 ± 6	69 ± 11	58 ± 7	48 ± 6	42 ± 5	42 ± 10	61 ± 15
Recombinant LSF	38 ± 7	60 ± 12	52 ± 9	48 ± 9	39 ± 6	38 ± 8	66 ± 25
Protein-free LSF	35 ± 6	60 ± 10	57 ± 10	56 ± 10	56 ± 10	61 ± 13	70 ± 9
Exosurf	38 ± 7	75 ± 13	64 ± 8	55 ± 6	48 ± 8	54 ± 11	93 ± 28

All values are given as mean ± standard deviation. $n = 10$-12 for each dose. The table shows the values before and after lavage as well as 5 and 120 min after instillation of 50 mg kg^{-1} body weight of the different LSF preparations. The last three columns show the values during the stepwise decrease of positive end-expiratory pressure (PEEP) to zero.

Table 3 Comparison of $Paco_2$ (mmHg) values after administration of 100 mg kg^{-1} body weight of Alveofact, Survanta, Recombinant LSF, protein-free, phospholipid-based LSF and Exosurf

	Before Lavage	*After Lavage*	*5 min after instillation of 100 mg kg^{-1} LSF*	*120 min after instillation of 100 mg kg^{-1} LSF*	*PEEP reductions 26/6*	*23/3*	*20/0*
Alveofact	40±9	70±9	61±6	56±6	46±7	38±6	45±10
Survanta	38±6	67±10	58±9	50±5	46±7	48±11	72±21
Recombinant LSF	44±9	63±10	53±7	46±11	39±8	39±10	48±11
Protein-free LSF	38±3	60±9	52±6	50±6	46±6	59±17	62±18
Exosurf	45±10	67±13	60±9	58±10	53±9	57±10	85±22

All values are given as mean ± standard deviation, $n = 10$-12 for each dose. The table shows the values before and after lavage as well as 5 and 120 min after instillation of 100 mg kg^{-1} body weight of the different LSF preparations. The last three columns show the values during the stepwise decrease of positive end-expiratory pressure (PEEP) to zero.

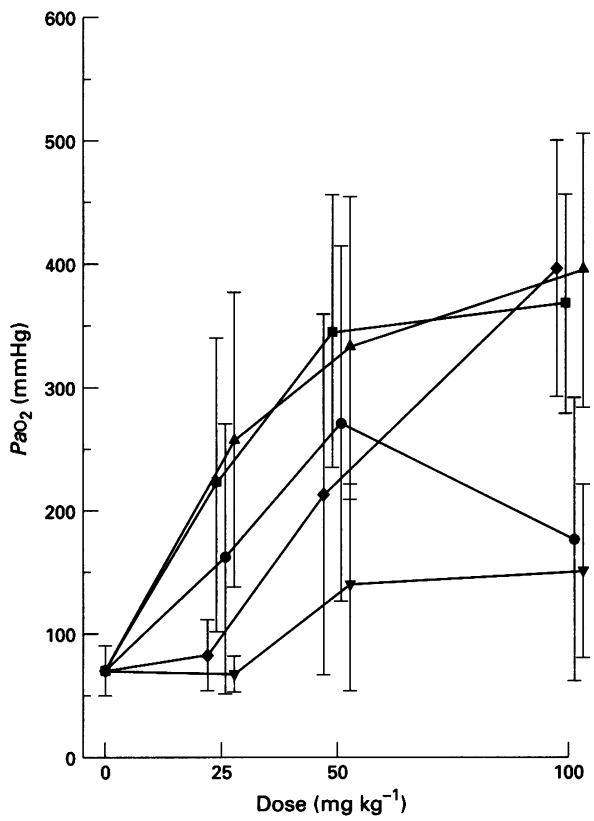

Figure 5 Dose response curves of Alveofact (◆); Survanta (■); Exosurf (●); protein-free, phospholipid-based LSF (▼) and Recombinant LSF (▲). The values represent mean ± s.d. based on the individual Pao_2 values during the 2nd PEEP reduction (PEEP = 3 cmH_2O) after administration of the different doses of the different LSF preparations.

fered significantly from Exosurf with $P \leqslant 0.001$ and Survanta differed significantly from Exosurf with $P \leqslant 0.01$. Recombinant LSF, Alveofact and Survanta differed significantly from PL-LSF with $P \leqslant 0.001$, whereas there was no statistically significant difference between PL-LSF and Exosurf.

This dose-response comparison shows that Recombinant LSF and Survanta behaved similarly after administration of the different doses, whereas Alveofact and Exosurf were different. Alveofact developed a clear dose-dependent efficacy with only little effect after administration of 25 mg kg^{-1} and after 100 mg kg^{-1} the efficacy was similar to Survanta or Recombinant LSF. Exosurf showed dose-dependency only after administration of the 25 and 50 mg kg^{-1} dose, whereas after administration of the 100 mg kg^{-1} dose the effect decreased. PL-LSF showed dose-dependency but was significantly worse than all protein-containing LSF preparations.

Comparison of the different doses of Exosurf to the control group showed that after administration of 25 mg kg^{-1} no significant difference was detectable, with a dose of 50 mg kg^{-1} this comparison resulted in significance ($P \leqslant 0.01$) but after a dose of 100 mg kg^{-1} the significance level decreased to $P \leqslant 0.05$. This was in contrast to the other LSF preparations, the two bovine LSF preparations showed an increasing significance level with increasing doses (Alveofact: 25 mg kg^{-1}, NS; 50 mg kg^{-1}, $P \leqslant 0.01$; and 100 mg kg^{-1}, $P \leqslant 0.001$; Survanta: 25 mg kg^{-1}, $P \leqslant 0.01$; 50 mg kg^{-1}, $P \leqslant 0.001$; and 100 mg kg^{-1}, $P \leqslant 0.001$). The protein-free PL-LSF showed a significant difference from controls only at a dose of 100 mg kg^{-1} ($P \leqslant 0.01$). However, all three doses of Recombinant LSF differed significantly with respect to the control group with $P \leqslant 0.001$.

Discussion

Using the rat lung lavage model, it is possible to characterize new synthetic LSF preparations in a standardized and systematic fashion (Lewis & Jobe, 1993) with commercially available surfactants. This was done by use of comparable doses of LSF with similar concentrations, the same mode of administration and the same volume for administration of the different LSF preparations. The results of this study on an ARDS model of pure surfactant depletion demonstrate that during ventilation with PEEP of 8 cmH_2O the two bovine, the Recombinant and the synthetic LSF preparations were almost equally effective but differed from the protein-free only phospholipid-based surfactant (PL-LSF); however, Exosurf showed highly variable activity (Figures 2, 3 and 4). The differences between the protein-free and the protein-containing LSF preparations were even more pronounced if dose-response comparisons were performed based on the Pao_2 values during the 2nd PEEP reduction. It is well known that PEEP by itself has beneficial effects on oxygenation (Stokke, 1976). Thus, if a moderately active LSF like Exosurf or PL-LSF is combined with ventilation under high PEEP, it is possible to re-establish gas exchange in the lung (Rider *et al.*, 1992). But the stabilization of the lungs due to LSF activity vanishes if the PEEP is reduced. We found that after administration of the different doses of both protein-free synthetic LSF preparations, the decrease by steps of PEEP to zero reduces the Pao_2 values to the level of the control group.

With respect to Exosurf, the results differ somewhat from the results of Cummings *et al.* (1992) and Ikegami *et al.* (1993) obtained with the pre-term lamb. Both groups have consistently shown that Exosurf did not improve oxygenation or lung mechanics in pre-term lambs. But there is at least one important difference between our data and those obtained by these other workers. We have used animals that are able to produce their own LSF i.e. the alveolar type II cells would be expected to have preformed LSF that can be secreted or can be released by the detergents that Exosurf contains. Another possible explanation regarding the slightly greater activity of

Exosurf in our experiments may be derived from the results of Ikegami *et al.* (1993). They have demonstrated that the activity of exogenous LSF can be improved after exposure to the preterm lung. These activation events are competing with inactivation phenomena within the lung to yield a net physiological effect. They assume that the balance probably depends on the surfactant used and the degree of maturation of the lung, which will influence endogenous surfactant availability (Ikegami *et al.*, 1993). Thus, this activation (or improvement) of Exosurf may be due to the association of exogenous surfactant with components of the endogenous surfactant. This may be more prominent in adult animals in which the superficial surfactant is washed out and the alveolar type II cells are intact, as can be concluded from the work of Ikegami *et al.* (1993). Surprisingly, this activation is more pronounced after Exosurf than after PL-LSF. But even this improvement by endogenous surfactant, whether through secretion of preformed LSF or stimulated synthesis of surfactant, vanishes during PEEP reduction. This activity also vanishes at the highest dose of Exosurf used (Figure 5) indicating that despite DPPC being one of the major components of surfactant (Lewis & Jobe, 1993), it is not solely responsible for the formation of an adequate surfactant layer in the lung and even an improved phospholipid matrix (as used with PL-LSF) is not able to achieve the same activity as that seen with the protein-containing LSF preparations. Taking these points together, the PEEP reduction manoeuvre serves as a good criterion when judging the real efficacy of LSF preparations.

Due to the differing behaviour during the 5–120 min period with constant PEEP and the differing dose-response characteristics between Recombinant LSF, Exosurf and the PL-LSF (that differs from Recombinant LSF only with respect to the surfactant protein C) but the lack of difference between Recombinant LSF and Survanta, we conclude that surfactant protein C (SP-C) is an important additive in achieving the same activity as bovine surfactants. Further evidence derives from data of Seeger *et al.* (1993) who have quantified the phospholipid as well as the surfactant protein B (SP-B) content of Alveofact and Survanta. Their data suggest that Alveofact is a SP-B driven LSF, whereas Survanta is principally a SP-C-based LSF. This is in good accordance with unpublished investigations from our department of molecular biology (personal communication Prof. Dr K.-P. Schäfer) showing that the SP-B and SP-C ratio of Alveofact is 2:1 and of Survanta is about 1 : 3. Seeger *et al.* (1993) have quantified the SP-B content of Survanta as $<0.25\%$ and that of Alveofact as $>1.5\%$ related to phospholipids. Concerning this essential role of lung surfactant proteins, our data further confirm that Recombinant LSF, an SP-C based surfactant, is equally as active as bovine LSF (Van Daal *et al.*, 1991). The failure of the synthetic LSF preparations Exosurf and PL-LSF to restore PaO_2 values after the PEEP reduction may be further explained by the observations of other authors who have found that LSF can be inhibited by certain proteins (Hallmann *et al.*, 1991; Kobayashi *et al.*, 1991). However, as Lachmann *et al.* (1994) have shown, normally, the inhibitory effects of plasma proteins can be neutralized by large amounts of an active exogenous surfactant, whereas in this work high concentrations of Exosurf had no additional beneficial effects. Furthermore, it is likely that active LSF preparations are able to influence transcapillary-interstitial-epithelial lung leakage (Lewis & Jobe, 1993) and can interfere with plasma proteins (Lachmann *et al.*, 1994). This is essential to prevent the formation of hyaline membranes (Enhörning, 1989; Nosaka *et al.*, 1990).

However, the present results of Exosurf are in good accordance with the results published so far after administration to ARDS patients (Wiedemann *et al.*, 1992). These authors were also unable to detect dose-dependent activity after administration of Exosurf. This is further evidence that an active LSF preparation should contain at least one surfactant protein. In addition, the recently published data of a large clinical trial with Exosurf (Anzueto *et al.*, 1994) and a pilot study using Survanta (Gregory *et al.*, 1994) provide further support. While Exosurf (Anzueto *et al.*, 1994) failed to show superior effects compared to conventional therapy, Survanta (Gregory *et al.*, 1994) showed statistically significant improvements. Thus, comparison of results from the rat lung lavage model described here with clinical data suggests that it is suitable for investigation of the effects and activity of LSF preparations that may be used for treatment of ARDS patients.

Values of $PaCO_2$ do not have such a great variablility as those of PaO_2 due to the administration of bicarbonate in our experiments, although they can normalize after administration of a very active LSF preparation and remain low during a decrease in PEEP (Tables 1–3). Again we have shown, even for this parameter, that LSF preparations that stabilize the lungs can tolerate a reduction of PEEP to zero. The results obtained in this specific model using the described ventilatory settings and a defined volume and dose of surfactant, suggest that protein-free surfactants based only on one phospholipid or a phospholipid mixture do not achieve the same activity as bovine LSF preparations. The conclusion that protein-containing LSF preparations are better than protein-free surfactants is based on the different dose-response curves (Figure 5) under ventilation with a PEEP of 3 cmH_2O (=2nd PEEP reduction, equivalent to about 150 min after LSF administration). However, the activity of such phospholipid-based LSF preparations can be enhanced by addition of surfactant protein C (SP-C). With s,s-dipalmitoylated SP-C identical to that of man it is possible to achieve the same activity as natural bovine LSF preparations. The advantage of such an LSF preparation is that an SP-C can be manufactured which is similar to human SP-C but in a way that keeps it free of all possible contaminants. Strict standards of quality control and analytical procedures can be applied throughout all steps of production to ensure that the amount of protein does not vary. In addition, the amounts of SP-C as well as all phospholipids will not be a limiting factor (Kendig *et al.*, 1988) in the manufacture of an active LSF preparation. Thus, it would solve one of the most frustrating problems in LSF research and treatment of ARDS patients, that is, the lack of sufficient amounts of LSF material for clinical trials and therapy.

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