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Surfactant Protein-B Supplementation Improves *In Vivo* Function of a Modified Natural Surfactant

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ABSTRACT

The effect of the addition of surfactant protein (SP)-B or SP-B plus SP-C (SP-BC) to a surfactant made from bovine lung (Survanta) was evaluated in 27-d-gestation preterm rabbits. The animals were treated with Survanta, Survanta + 2% SP-B, Survanta + 3% SP-BC, or sheep surfactant. They were then ventilated with 3 cm H_2O positive end-expiratory pressure and tidal volumes of 8 mL/kg. Survanta + 2% SP-B was prepared by adding SP-B in water to Survanta or by adding SP-B in chloroform [SP-B(Chl)] to lipid-extracted Survanta. Dynamic compliances of the Survanta + 2% SP-B(Chl)- and Survanta + 3% SP-BC-treated rabbits were greater ($p < 0.05$) than those treated with Survanta or Survanta + 2% SP-B in water and were comparable to sheep surfactant. Postventilation pressure-volume curves for the groups treated with Survanta supplemented with SP-B had significantly larger retained volumes on deflation compared with those treated only with Survanta ($p < 0.01$). The effects of ventilation style on the responses were assessed by ventilating other groups of rabbits treated with Survanta, Survanta + 0.5% SP-B(Chl), Survanta + 2% SP-B(Chl), or sheep surfactant with tidal volumes of 10 mL/kg and 0 cm H_2O positive end-expiratory pressure. SP-B (2%) augmented the *in vivo* function of Survanta without positive end-expiratory pressure, and 0.5% SP-B had no effect. Infasurf, a surfactant with more SP-B, was more effective than Survanta when tested in the preterm rabbits. SP-B is a critical factor for optimum immediate surfactant function. (***Pediatr Res* 37: 271–276, 1995**)

Abbreviations

SP-B, surfactant protein-B
SP-C, surfactant protein-C
SP-A, surfactant protein-A
SP-B(H_2O), SP-B in water
SP-B(Chl), SP-B in chloroform
SP-BC, mixture of SP-B and SP-C
PEEP, positive end-expiratory pressure
PIP, peak inspiratory pressure

Natural surfactant as recovered by lavage from the air spaces of healthy mammalian lungs is a complex of lipids and specific proteins (1). The lipophilic proteins SP-B and SP-C are critical for surface adsorption and low surface tensions (2–4), and SP-A interacts cooperatively with the other surfactant proteins to influence surface properties (5). In general, lipid-only surfactants are much less effective in preterm surfactant-deficient lungs or surfactant-depleted lungs than natural surfactants that contain SP-B or SP-C (6, 7). Several lines of evidence indicate that SP-B is critical to short-term surfactant efficacy. MAb to SP-B induce severe respiratory failure, and SP-B deficiency in term infants results in pulmonary death (8, 9). Rider *et al.* (10) recently demonstrated that the addition of SP-B to natural surfactant lipids yielded a surfactant that was as effective as natural sheep surfactant when preterm rabbits were ventilated with PEEP. However, in the absence of PEEP, the lipids plus SP-B were not as effective as natural surfactant.

The lung-derived surfactants used clinically are made from organic solvent extracts of saline extracts of minced lung or from organic solvent extracts of alveolar lavage (11). These surfactants contain the lipophilic proteins SP-B and SP-C in varying amounts that have not been accurately quantified. SP-A is removed by the extraction procedures that also disrupt the lipoprotein structural arrays. In several test systems in surfactant-deficient lungs *in vivo*, Survanta, a surfactant made from bovine lungs, was less effective at improving gas exchange, pressure-volume curves, or compliance than natural surfactant or organic solvent extracts of calf lung lavage, often referred to as calf lung surfactant extract (6, 12–14). We asked whether the acute function of Survanta could be improved by supplementation with the lipophilic surfactant proteins.

METHODS

Surfactants

Survanta (Ross Laboratories, Columbus, OH) is prepared by the organic solvent extraction of saline extracts of minced

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bovine lung. The lipid extracts then are supplemented with dipalmitoylphosphatidylcholine, palmitic acid, and tripalmitin to enhance *in vitro* surface properties (15). Infasurf (ONY, Buffalo, NY; provided by Dr. Ted Egan) is a chloroform-methanol extract of calf lung lavage (14, 16). Natural sheep surfactant was recovered from fresh adult sheep lungs by differential centrifugation as reported previously (17).

Isolation of SP-B and SP-BC

SP-B was isolated from sheep surfactant by differential solvent extraction as described in detail by Bates *et al.* (18). Briefly, aliquots of surfactant were delipidated by extraction three times with isopropyl ether-butanol (3:2, vol/vol). The aqueous layer was dried and sequentially extracted with ethyl ether-ethanol (3:1, vol/vol) and with chloroform-methanol-0.005N HCl (3:2, vol/vol). SP-B in the chloroform-methanol solvent was dried by rotary evaporation and resuspended in distilled water by shaking manually with glass beads. The pH was adjusted to 7.0 with NaOH. The protein content was determined by the Lowry method (19) modified by the addition of 1% SDS, and BSA was used as the standard (20). The purity of isolated SP-B was verified on 15% SDS-polyacrylamide gels run with 10% 2-mercaptoethanol. A silver stain was used to visualize the proteins. SP-B(H_2O) was mixed with Survanta to a final ratio of 2% by weight and incubated at 37°C for 2 h before testing in preterm rabbits. Part of SP-B(H_2O) was dissolved in chloroform by recovering chloroform phase after extraction with chloroform-methanol-water (3:2:1, vol/vol/vol). Survanta was extracted with chloroform-methanol-water (2:1:1, vol/vol/vol), and SP-B(Chl) was added at the ratio of 2% or 0.5% by weight. The mixture was dried by rotary evaporation at 50°C, and suspensions in 0.9% NaCl were prepared by shaking the flask with glass beads.

SP-BC was isolated from organic solvent extracts of sheep surfactant on Sephadex LH-20 columns (Pharmacia Fine Chemicals Ltd., Uppsala, Sweden) equilibrated with chloroform-methanol (2:1, vol/vol) (2, 21, 22). The effluent was monitored at 280 nm, and fractions from the first two thirds of the first protein peak were combined. After protein content was measured, the SP-BC in chloroform-methanol was added to organic solvent–extracted Survanta at the ratio of 3:100 by weight and then dried with a rotary evaporator. Dried Survanta plus SP-BC was suspended in 0.9% NaCl by shaking manually with glass beads.

The final concentrations for the surfactants used in the surfactant protein addition experiments were adjusted to 18 mg total lipids plus protein/mL. All surfactants were given to preterm rabbits at a dose of 100 mg/kg body weight (5.5 mL/kg). For the comparison experiment of Survanta and Infasurf, the surfactants were given at the clinically recommended doses of 4 mL/kg (100 mg total lipids/kg) for Survanta or 3 mL/kg (105 mg total lipids/kg) for Infasurf.

Surfactant Testing *In Vivo*

Surfactant-deficient, 27-d-gestational-age premature rabbits were used to test *in vivo* function as described previously (6, 12). In brief, the preterm rabbits were sequentially delivered from anesthetized does and anesthetized with an intraperitoneal injection of a mixture of 10 mg/kg ketamine and 0.1 mg/kg acepromazine. The trachea of each rabbit was cannulated, and one of the surfactant suspensions was given via the tracheal tube. A control group was untreated. Rabbits were ventilated for 15 min in a series of plastic, 37°C temperature-controlled ventilator plethysmographs (23) with 100% oxygen at rate of 30 breaths/min with an inspiratory time of 1 s and PEEP of 3 cm H_2O or 0 cm H_2O. PIP were individually regulated to adjust the tidal volume as measured with a pneumotachometer to 8 mL/kg with 3 cm H_2O PEEP and 10 mL/kg with 0 cm H_2O PEEP. These combinations of tidal volumes and PEEP resulted in normal Pco_2 values in 27-d-gestational-age preterm rabbits (6). Dynamic compliance was calculated by dividing total volume per kg body weight by PIP – PEEP. At 15 min of ventilation, high-speed paper recordings (25 mm/s) were obtained for three ventilatory cycles for assessment of the expiratory time constant from the tidal volume recordings. The value of expiratory time constant was the time that was required for lung volume to fall to 37% of its maximum volume (24, 25). The endotracheal tube then was plugged for 5 min to allow absorption atelectasis to occur, and quasi-static pressure-volume curves were measured (4, 21).

Treatment Protocols

Ventilation with 3 cm H_2O PEEP. Eight litters of rabbits were delivered and treated with Survanta, Survanta + 2% SP-B(H_2O), Survanta + 2% SP-B(Chl), Survanta + 3% SP-BC, or sheep surfactant. Each litter contained at least one untreated control animal. The animals were treated in a continuous sequence across litters so that birth order was balanced for the treatment groups. The rabbits then were ventilated with 3 cm H_2O PEEP, and tidal volumes were adjusted to 8 mL/kg.

Ventilation with 0 cm H_2O PEEP. Previously, we found that some surfactants are less effective if preterm rabbits are ventilated without PEEP (10). Therefore, five litters were delivered and treated with Survanta, Survanta + 0.5% SP-B(Chl), Survanta + 2% SP-B(Chl), or sheep surfactant, or they were left untreated. These animals were ventilated to achieve tidal volumes of 10 mL/kg body weight.

Comparison of Survanta and Infasurf. Additional rabbits were randomized to receive Survanta, Infasurf, or no treatment. These animals then were divided into groups for ventilation with and without PEEP.

Amounts of SP-B and SP-C in the Surfactants

The amounts of SP-B and SP-C contained in Survanta and Infasurf were measured by the Lowry method modified by the addition of 1% SDS after delipidation by extraction three times with 10 mL of isopropyl ether-butanol (3:2, vol/vol). The aqueous layer of sheep surfactant obtained after extraction with isopropyl ether-butanol was further extracted two times with chloroform-methanol-0.005N HCl (3:2, vol/vol) to remove hydrophilic proteins. The recovered chloroform-methanol solution was dried and resuspended in 1% SDS for protein measurement.

Data Analysis

All values are expressed as mean ± SEM. Differences in means between groups were tested by analysis of variance followed by the Student-Newman-Keuls multiple comparison procedure. Significance was accepted at $p < 0.05$.

RESULTS

Ventilation with 3 cm H_2O PEEP. The rabbits in the different surfactant treatment groups had similar birth weights and tidal volumes at 15 min of age (Table 1). The ventilatory pressure requirements (PIP – PEEP) of rabbits treated with Survanta + 2% SP-B(Chl) or Survanta + 3% SP-BC at 15 min of age were similar to the sheep surfactant-treated group and significantly less than rabbits treated with Survanta. However, ventilatory pressure requirements of rabbits treated with Survanta + 2% SP-B(H_2O) were higher than the Survanta + 2% SP-B(Chl) group and were similar to the Survanta group. The expiratory time constants for the Survanta + 2% SP-B(Chl) or Survanta + 3% SP-BC groups were longer than for the Survanta group and were similar to the sheep surfactant group. The expiratory time constant for rabbits treated with Survanta + 2% SP-B(H_2O) was not different from the Survanta group.

Survanta improved dynamic compliance at 15 min (Fig. 1*A*) and pressure-volume curves (Fig. 1*B*) when compared with the control group. However, the responses were significantly less than those for sheep surfactant. Addition of 2% SP-B(Chl) or 3% SP-BC improved dynamic compliances from that measured with Survanta ($p < 0.05$). However, addition of 2% SP-B(H_2O) did not improve dynamic compliance from that measured with Survanta. The compliance of the 2% SP-B(H_2O) group was less than with Survanta + 2% SP-B(Chl) and sheep surfactant ($p < 0.05$). The addition of SP-B(Chl), SP-B(H_2O), or SP-BC to Survanta increased lung volumes at 10 or 5 cm H_2O on the deflation limbs of the pressure-volume curves compared with Survanta ($p < 0.05$). The curves for the Survanta + 2% SP-B group and Survanta + 3% SP-BC group were similar to that for the sheep surfactant group. For all the measurements made with 3 cm H_2O PEEP, addition of 2% SP-B(Chl) or 3% SP-BC enhanced the function of Survanta to be equivalent to that of sheep surfactant. On the other hand, addition of 2% SP-B(H_2O) did not improve dynamic compliance or the expiratory time constant at 15 min of ventilation, indicating that the method of addition of SP-B to Survanta was important.

Ventilation with 0 cm H_2O PEEP. There were no significant differences in body weights or tidal volumes at 15 min between groups (Table 2). The ventilatory pressure requirement at 15 min of age for rabbits treated with Survanta + 2% SP-B was similar to that for sheep surfactant and significantly less than for Survanta and Survanta + 0.5% SP-B. There were no significant differences in the expiratory time constant values at 15 min between the Survanta + 2% SP-B group and the Survanta group.

Dynamic compliance (Fig. 2*A*) of rabbits at 15 min treated with Survanta + 2% SP-B was significantly greater than compliances in the control and Survanta groups and similar to that in the sheep surfactant group. The response to Survanta + 0.5% SP-B was similar to the response to Survanta and significantly less than responses to Survanta + 2% SP-B or to sheep surfactant. The pressure-volume curve for Survanta + 2% SP-B was improved compared with those for the Survanta,

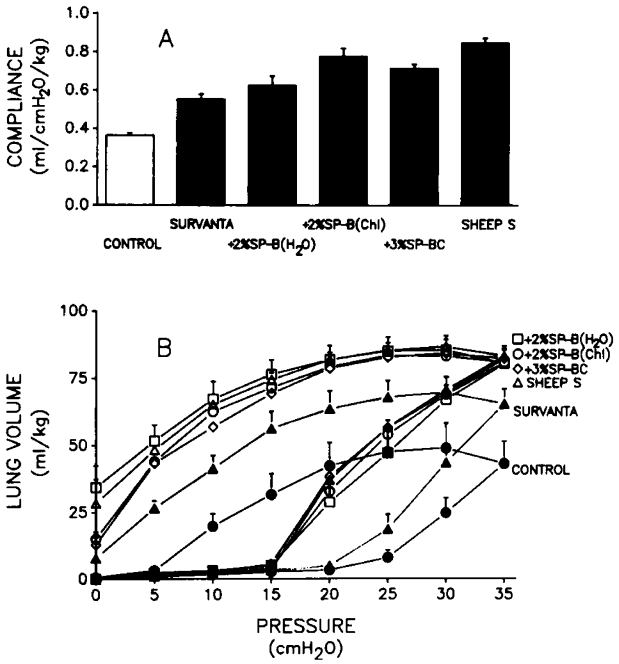

Figure 1. Compliance (*A*) and pressure-volume curves (*B*) for preterm rabbits ventilated with 3 cm H_2O PEEP. The study groups were untreated controls (group 1) and rabbits treated with Survanta (group 2), Survanta + 2% SP-B(H_2O) (group 3), Survanta + 2% SP-B(Chl) (group 4), Survanta + 3% SP-BC (group 5), and sheep surfactant (group 6). Differences in dynamic compliances at 15 min of ventilation were groups 4 and 6 > 2 > 1 ($p < 0.01$), group 5 > 2 ($p < 0.05$), and group 6 > 3 ($p < 0.05$). Differences in lung volumes on pressure-volume curves were as follows: volume at 35 cm H_2O: groups 2, 3, 4, 5, 6 > 1 ($p < 0.05$); volume at 10 cm H_2O: groups 3, 4, 6 > 2 > 1 ($p < 0.01$), group 5 > 2 ($p < 0.05$); and volume at 5 cm H_2O: groups 3, 4, 5, 6 > 2 > 1 ($p < 0.01$).

Table 1. *Ventilation with 3 cm H_2O PEEP*

Treatment group	*n*	Body wt (g)	Tidal volume (mL/kg)	PIP – PEEP (cm H_2O)	Expiratory time constant (s)
1. Control	7	28.7 ± 1.1	8.3 ± 0.1	23.0 ± 0.9	0.32 ± 0.01
2. Survanta	7	28.8 ± 1.4	8.1 ± 0.1	14.8 ± 0.8	0.38 ± 0.01
3. Survanta + 2% SP-B(H_2O)	11	31.2 ± 1.4	8.3 ± 0.1	14.1 ± 1.2	0.42 ± 0.01
4. Survanta + 2% SP-B(Chl)	10	32.8 ± 1.6	8.3 ± 0.1	10.9 ± 0.6	0.48 ± 0.02
5. Survanta + 3% SP-BC	7	31.0 ± 1.7	8.3 ± 0.1	11.6 ± 0.4	0.45 ± 0.01
6. Sheep surfactant	6	29.5 ± 0.9	8.4 ± 0.1	10.0 ± 0.4	0.45 ± 0.01

PIP-PEEP, groups 4, 5, and 6 < 2 < 1 and 4 and 6 < 3 ($p < 0.05$); Expiratory time constant, groups 4, 5, and 6 > 2 > 1 and 4 > 3 ($p < 0.05$).

Table 2. *Ventilation with 0 cm H_2O PEEP*

Treatment group	*n*	Body wt (g)	Tidal volume (mL/kg)	PIP – PEEP (cm H_2O)	Expiratory time constant (s)
1. Control	8	28.8 ± 3.6	10.2 ± 0.1	30.5 ± 0.9	0.34 ± 0.01
2. Survanta	8	29.2 ± 3.4	10.2 ± 0.1	23.5 ± 0.6	0.38 ± 0.01
3. Survanta + 0.5% SP-B	8	29.4 ± 5.1	10.3 ± 0.1	23.5 ± 0.8	0.41 ± 0.01
4. Survanta + 2% SP-B	8	28.3 ± 3.4	10.4 ± 0.1	20.2 ± 0.6	0.42 ± 0.01
5. Sheep surfactant	7	29.5 ± 3.1	10.4 ± 0.1	19.2 ± 1.6	0.43 ± 0.02

PIP-PEEP, groups 4 and 5 > 2 and 3 > 1 ($p < 0.05$); Expiratory time constant, groups 2, 3, 4, and 5 > 1 ($p < 0.01$), 5 > 2 ($p < 0.05$).

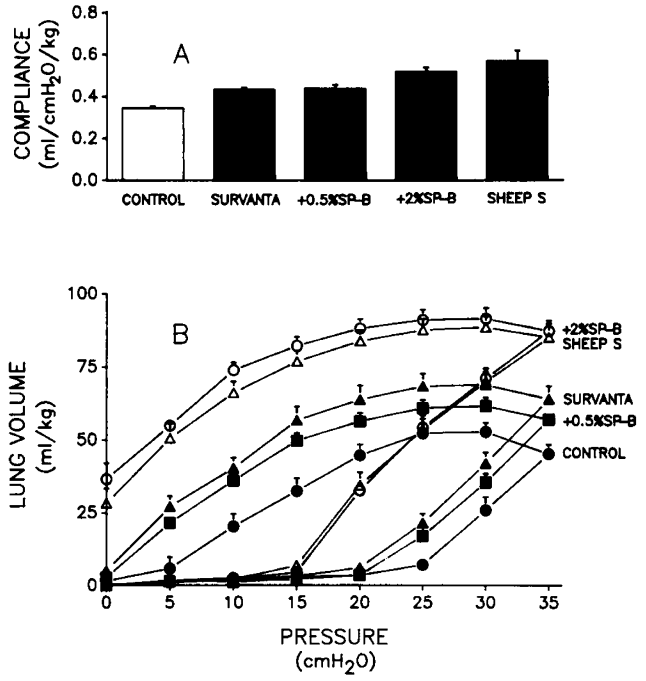

Figure 2. Compliances (*A*) and pressure-volume curves (*B*) for preterm rabbits ventilated without PEEP. The study groups were untreated controls (group 1), and rabbits treated with Survanta (group 2), Survanta + 0.5% SP-B (group 3), Survanta + 2% SP-B (group 4), and sheep surfactant (group 5). Differences in dynamic compliances at 15 min of ventilation were groups 4 and 5 > 2 and 3 > 1 ($p < 0.05$). Differences in lung volumes on pressure-volume curves were as follows: volume at 35, 10, 5 cm H_2O on deflation: groups 4 and 5 > 2 and 3 > 1 ($p < 0.01$).

Survanta + 0.5% SP-B, and control groups (Fig. 2*B*). The opening pressures for the sheep surfactant and Survanta + 2% SP-B groups were 15 cm H_2O. The lungs treated with Survanta or Survanta + 0.5% SP-B and the lungs left untreated began to open at approximately 25 cm H_2O. The volumes at 35 cm H_2O for sheep surfactant and Survanta + 2% SP-B groups were 85.5 and 87.7 mL/kg, respectively, and were significantly greater than those of the Survanta, Survanta + 0.5% SP-B, and control groups. Sheep surfactant and Survanta + 2% SP-B groups had larger volumes at 5 and 10 cm H_2O on the deflation limbs than Survanta, Survanta + 0.5% SP-B, and the control group.

Comparison of Survanta and Infasurf. In the presence of PEEP, ventilatory pressure requirements at 15 min of the Survanta group were not different from those of the Infasurf group (Table 3). However, in the absence of PEEP, the ventilatory pressures needed for the Survanta group were higher than those for the Infasurf group. The expiratory time constant at 15 min of age for the Survanta group was lower than that for the Infasurf group ventilated with or without PEEP. The dynamic compliance response to Survanta was lower than the response to Infasurf in the absence of PEEP (Fig. 3*A*). However, with 3 cm H_2O PEEP, the dynamic compliance response with Survanta was comparable to the response to Infasurf and was significantly greater than the compliance in the control group. The pressure-volume curves for the animals that were ventilated with 0 cm H_2O were not different from the curves for animals ventilated with 3 cm H_2O PEEP. Therefore, mean curves for all ventilated animals within a surfactant treatment group were calculated (Fig. 3*B*). The lung volume at 35 cm H_2O for Infasurf was higher than that for Survanta. The same pattern was found at 10 and 5 cm H_2O pressure on the deflation limbs of the pressure-volume curves.

Amounts of SP-B or SP-BC. Tricine SDS polyacrylamide gels (26) run with equal amounts of the delipidated surfactant samples indicated qualitatively that Survanta contained less SP-B than Infasurf or sheep surfactant (data not shown). The amount of total protein, presumably SP-BC, in each 1 mg of lipid extract as estimated by protein assay was 8.0 ± 0.14 μg (Survanta, $n = 11$), 18.6 ± 0.2 μg (Infasurf, $n = 11$), and 19.4

Table 3. *Comparison of Survanta and Infasurf*

Treatment group	*n*	Body wt (g)	Tidal volume (mL/kg)	PIP – PEEP (cm H_2O)	Expiratory time constant (s)*
Ventilation with 3 cm H_2O PEEP					
1. Control	4	28.1 ± 1.7	8.5 ± 0.2	24.0 ± 1.2†	0.34 ± 0.01
2. Survanta	5	31.1 ± 2.6	8.1 ± 0.1	14.0 ± 1.0†	0.38 ± 0.01
3. Infasurf	5	31.1 ± 1.1	7.9 ± 0.3	12.3 ± 1.7†	0.45 ± 0.01
Ventilation with 0 cm H_2O PEEP					
1. Control	4	29.0 ± 1.5	9.9 ± 0.3	28.1 ± 0.6‡	0.30 ± 0.01
2. Survanta	10	29.4 ± 1.1	10.1 ± 0.1	24.0 ± 0.6‡	0.36 ± 0.01
3. Infasurf	10	30.6 ± 0.7	10.1 ± 0.1	20.0 ± 0.7‡	0.40 ± 0.01

* Group 3 > 2 > 1 ($p < 0.05$).
† Groups 2 and 3 < 1 ($p < 0.01$).
‡ Group 3 < 2 < 1 ($p < 0.01$).

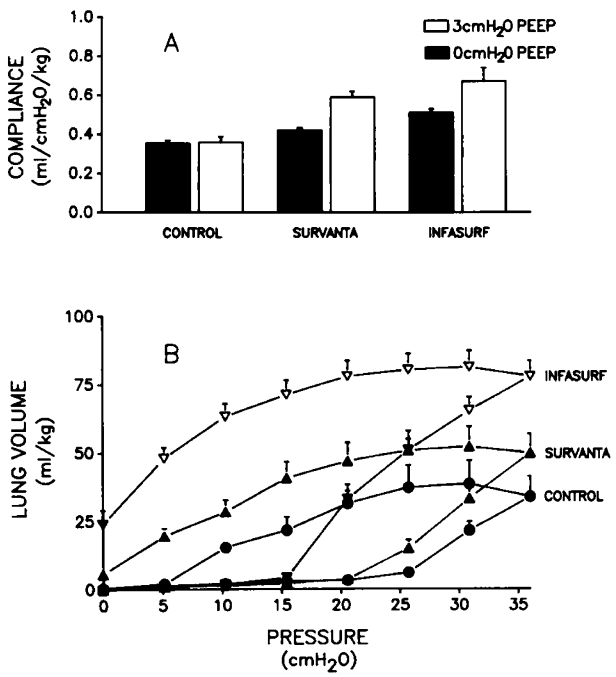

Figure 3. Compliance (*A*) and pressure-volume curves (*B*) for Survanta- and Infasurf-treated preterm rabbits ventilated with or without PEEP. The study groups were untreated controls (group 1) and rabbits treated with Survanta (group 2) or Infasurf (group 3). Pressure-volume curves are composit curves for animals studied with and without PEEP. Differences in dynamic compliances at 15 min of ventilation were as follows: with 3 cm H_2O PEEP: groups 2 and 3 > 1 ($p < 0.01$); with 0 cm H_2O PEEP; group 3 > 2 > 1 ($p < 0.05$). Differences in lung volumes on pressure-volume curves were as follows: volumes at 35 and 10 cm H_2O on deflation: group 3 > 1 and 2 ($p < 0.01$); volumes at 5 cm on deflation: group 3 > 2 > 1 ($p < 0.01$).

± 0.2 μg (sheep surfactant, $n = 11$). The total protein contained in Survanta was significantly less than the amount in the other surfactants ($p < 0.01$).

DISCUSSION

Addition of 2% SP-B or 3% SP-BC(Chl) to Survanta augments its short-term *in vivo* performance to be equivalent to that of sheep surfactant. All indicators of function became essentially equivalent to those of sheep surfactant. Compliance improved with or without PEEP, expiratory time constants became longer, and pressure-volume curves were superimposable. *In vivo* function of lipid-extracted Survanta was not different from that of Survanta (data not shown). These results demonstrate that there is insufficient SP-B in Survanta for optimal function immediately after treatment. Seeger *et al.* (27) reported that Survanta contained <0.1% SP-B, and surfactants made by organic solvent extracts of lavage surfactant contain >1% SP-B. There are several reasons why Survanta contains less SP-B than sheep surfactant or Infasurf, an organic extract of alveolar lavage. The saline extraction of minced lungs followed by organic solvent extraction probably recovers less SP-B relative to other surfactant components. The subsequent supplementation with synthetic lipids will further decrease the SP-B content. There have been no studies on the stability of the functional properties of SP-B with organic solvent extraction. Natural sheep surfactant and Survanta are functionally equivalent when Survanta is enhanced by adding 2% purified SP-B; this effect is not noted after addition of only 0.5% SP-B.

The amount of SP-B or SP-C in any surfactant has not been accurately measured because reliable assays are not available. Gel electrophoresis can be used as a qualitative assessment, but the traditional protein assays are not accurate in the presence of large amounts of lipid. If the surfactants are delipidated, there may be loss of some lipophilic protein. These proteins must then be assayed in the presence of detergents. The percentages of SP-B used in these supplementation experiments must be recognized to be approximations of the true content. For example, we measured SP-B by a modified Lowry assay and used that value for supplementation. When samples of our SP-B were evaluated by an ELISA for SP-B, the content was estimated to be 1.4-fold higher (28, 29). Survanta previously was reported to contain about 1% protein that is SP-BC (30). Our estimate on delipidated samples is 0.8%. Bates *et al.* (31) used a type II cell binding assay to estimate that the amount of SP-B in a calf lavage lipid extract was about 1.5% by weight. Infasurf also is thought to contain about 2% SP-BC (14). We also found that the way SP-B is added to a surfactant can be critical. Addition of SP-B(H_2O) improved the pressure-volume curve, but it did not improve compliance measured *in vivo* or lengthen the expiratory time constant. Although we do not know the reason for this partial effect, the addition of SP-B(H_2O) caused the loss of the dynamic effects of the SP-B supplementation resulting from mixing with chloroform. Although SP-B(H_2O) was found to bind to type II cells (31), mixing of the lipids and SP-B(Chl) was required for optimal enhancement of *in vivo* function.

An interesting finding was that supplementation of Survanta with 2% SP-B enhanced function even when the preterm rabbits were ventilated without PEEP. We previously found that addition of SP-B, SP-C, or SP-BC to natural surfactant lipids did not restore surfactant function to that function achieved with natural sheep surfactant. Some factor or method of recombination was still missing. SP-B alone reversed the sensitivity of Survanta to ventilation without PEEP. Infasurf performed in the absence of PEEP and therefore is more like natural sheep surfactant. The hydrophilic surfactant protein SP-A did not augment the function of Survanta (12).

These results are consistent with the critical role of SP-B in surfactant function. Synthetic and natural lipid mixtures are much less effective as surfactant than natural surfactant or surfactant lipids supplemented with SP-B or SP-BC. Antibodies to SP-B can cause severe respiratory failure, and a genetic deficiency of SP-B causes lethal respiratory failure in term infants. An SP-C deficiency disease has not been identified. The principal surfactant in use clinically does not have enough SP-B for optimal acute function. However, this and other surfactants are effective in part because they provide substrate for the normal metabolic pathways in the preterm lung. Ikegami *et al.* (7) recently reported that clinical surfactants can be activated in the preterm lamb lung. This improvement in function may be explained by the entrance of endogenously produced surfactant proteins into the lipids used for treatment.

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