Dexamethasone Treatment of Infants at Risk for Chronic Lung Disease: Surfactant Components and Inflammatory Parameters in Airway Specimens

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ABSTRACT

The mechanisms explaining the beneficial effects of glucocorticoid in ventilator-dependent preterm infants are not known. In the present randomized trial, we evaluated the hypothesis that dexamethasone (DEX) treatment of small, preterm infants at risk for chronic lung disease favorably affects the surfactant system. Twenty-three ventilator-dependent infants, with a mean $\pm$ SD gestational age of 26 ± 2 wk and a mean birth weight of 836 ± 173 g, received 1 wk of treatment with either DEX (dose 0.5 mg/kg/d) or placebo beginning at 2 wk of age. The airway specimens were analyzed for surfactant components, surface activity, surfactant inhibitors, and inflammatory mediators. The concentrations of these parameters in epithelial lining fluid were calculated using the urea method. DEX treatment decreased the concentration of nonsedimentable protein in epithelial lining fluid within 3 d ($p < 0.05$). The nonsedimentable fraction of airway specimens decreased the surface activity of surfactant as a function of protein concentration. At a constant protein concentration, the protein from placebo-treated infants inhibited the surface activity of human surfactant *in vitro* more than protein from DEX-treated infants ($p < 0.05$). DEX transiently increased the

concentration of surfactant protein-A in epithelial lining fluid but had no effect on surface activity of the sedimentable surfactant complex or on concentrations of phosphatidylcholine, IL-1 β , lactoferrin, or myeloperoxidase. We conclude that the acute beneficial effect of DEX treatment in preterm ventilator-dependent infants may in part be mediated through a decrease in the concentration of non-sedimentable protein and a decrease in the capacity of this protein to inhibit surface activity. (*Pediatr Res* 36: 387–393, 1994)

Abbreviations

AS, airway specimen
CLD, chronic lung disease
DEX, dexamethasone
ELF, epithelial lining fluid
LF, lactoferrin
MPO, myeloperoxidase
PC, phosphatidylcholine
PL, placebo
SP-A, surfactant protein-A
SPC, disaturated phosphatidylcholine

Despite improved survival, many preterm infants fail to recover from the acute lung injury and develop CLD. In addition to immaturity, factors contributing to the development of CLD include barotrauma, oxygen toxicity, and inflammation (1, 2). The significance of the various suggested pathogenetic mechanisms is unclear.

DEX therapy decreases the requirement of respiratory support and improves pulmonary mechanics in ventilator-dependent preterm infants (3–6). In several controlled

trials on ventilator-dependent very-low-birth-weight infants, DEX therapy has improved the short-term pulmonary outcome, decreasing the duration of mechanical ventilation, but not the long-term outcome (7–11). Many potential mechanisms for the acute effect of DEX have been proposed. These include an increase in surfactant synthesis (12, 13) and in activity of antioxidant enzymes (14) shown after prenatal glucocorticoid therapy in fetal animals, and reduction of pulmonary edema, lung water, and alveolar epithelial permeability shown in preterm infants with CLD (5, 6, 15). DEX may additionally decrease pulmonary inflammation, suggested by reduction of neutrophil recruitment into the lungs (5, 16), and decrease bronchoalveolar lavage fluid concentrations of

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elastase (5, 16, 17). However, the precise mechanism of action of DEX in ventilator-dependent, small, preterm infants is not known.

We performed a randomized PL-controlled multicenter trial on very-low-birth-weight infants, who were ventilator and oxygen dependent at the age of 10 d (18). Within the context of the trial, our aim was to investigate whether administration of DEX was associated with changes in inflammatory indices, surfactant components, or surface activity in the ELF and whether any of these parameters correlated with the pulmonary outcome.

METHODS

Patients. The 23 infants studied were treated at the Children's Hospital, University of Helsinki, between January 1989 and March 1991. They participated in the randomized, multicenter study of DEX therapy in infants at risk for CLD and composed 56% of all infants participating in this study (18). AS were available from all infants who were recruited into the study at the Children's Hospital, University of Helsinki. The very-low-birth-weight infants were eligible for the study if they were ventilator dependent at 10 d of age and had no major malformation, sepsis, or patent ductus arteriosus. After informed consent was obtained from the parents, infants were randomly allocated to receive a 7-d course of either DEX sodium phosphate (Oradexon, Organon, Oss, The Netherlands) or PL. Infants in the DEX group received 0.5 mg/kg/d DEX i.v. divided into two doses and those in the PL group received 0.9% saline. The study design was approved by the ethical committee of the hospital.

All infants required ventilatory support from birth. For all infants, supplemental oxygen and mechanical ventilation were adjusted to maintain arterial P_{O_2} between 6.5 and 9.1 kPa (50–70 mm Hg) or oxygen saturation of Hb between 88 and 92% and arterial or capillary P_{CO_2} between 5.8 and 7.8 kPa (45–60 mm Hg). Ventilatory support was provided with a pressure-limited ventilator (Baby-Bird, Bird Corp., Palm Springs, CA). Pulmonary outcome was evaluated at 28 d of age. CLD was defined as requirement of supplemental oxygen at 28 d.

Collection of the AS. Tracheal aspirates of the intubated infants were obtained 1 to 2 d before the trial medication was started and daily during the 7-d treatment period at times when suctioning was deemed necessary by the nursing staff. Before suctioning, 0.9% saline (0.5 to 1.0 mL) was instilled into the intubation tube, and the infant was ventilated during a brief period. A catheter was then introduced into the airway distal to the tip of the endotracheal tube. Any fluid from the airways was suctioned into a mucus extractor (UnoPlast AS, Hundested, Denmark) within less than 20 s after the introduction of saline into the airways. After the procedure, 0.5 to 1.0 mL of saline was suctioned through the catheter into the Leukens trap to recover material retained in the side walls. Aspirates containing visible blood were not analyzed. The specimens were stored for a maximum of 12 h at 4°C

and centrifuged at $150 \times g$ for 10 min. The resulting supernatant, called AS, was recovered and stored at -20°C . A fraction (0.2 to 1.5 mL) of the AS was further centrifuged at $40\,000 \times g$ for 120 min, and the new high-speed supernatant was used for analysis of nonsedimentable protein and urea (19). The surfactant-rich lipid-protein complex, obtained as a pellet after the centrifugation at $40\,000 \times g$ for 120 min, was washed once by suspension in water before resedimentation.

Measurements of proteins, phospholipids, and surface tension. SP-A was quantified in AS using a two-site immunoassay with two different MAb. The method has been described in detail previously (20, 21). Total nonsedimentable protein was measured by a modified Lowry method (22). Protein electrophoresis was performed in 10% SDS polyacrylamide gel with Mini-Protean II Cell (Bio-Rad Laboratories, Richmond, CA). Ratios of protein in AS to serum protein are presented for infants whose serum protein samples were collected within 24 h of the AS. The concentration of total PC and SPC in AS was measured as described (19).

The measurements of surface tension were performed at 37°C in humidified air in 150 mmol/L NaCl, 1.5 mmol/L CaCl_2 , and 10 mmol/L Tris-Cl (pH 7.4). The pulsating bubble surfactometer was used (23). The bubble radius of 0.40 mm was maintained for 15 s. Thereafter, the radius was varied between 0.40 and 0.55 mm at a frequency of 0.33 Hz for 3 min. The pressure difference (ΔP) across the surface was continuously recorded. The surface tension at minimum bubble radius was calculated according to the law of Young and LaPlace: $\Delta P = 2\gamma/\text{radius}$. The rate of surface adsorption was estimated as follows: the bubble (radius 0.4 mm) was created and maintained while the decrease of surface tension was continuously recorded until it reached a plateau (*i.e.* equilibrium surface tension). The rate of surface adsorption illustrates the decrease of surface tension from 70 mN/m toward equilibrium surface tension.

AS was studied for its components and its capacity to decrease surface activity. The surfactant-rich fraction was isolated by differential sedimentation as described, and the phospholipid concentration of the lyophilized material was adjusted to 2 $\mu\text{mol/mL}$ of 150 mmol/L NaCl, 1.5 mmol/L CaCl_2 , 10 mmol/L Tris-Cl (pH 7.4). For the measurement of the surfactant inhibitor, the high-speed supernatant ($40\,000 \times g$ for 120 min) was further fractionated using a Centricon 3 microconcentrator (cutoff 3 kD; Amicon, Beverly, MA). The protein-rich retentate was lyophilized. The protein was adjusted to a final concentration of 4 or 8 g/L: the dry specimen was brought into suspension by adding 150 mmol/L NaCl, 1.5 mmol/L CaCl_2 , 10 mmol/L Tris-Cl (pH 7.4). Thereafter, human surfactant isolated from the amniotic fluid (24) was added to a final concentration of 1.8 or 7.2 mmol PC/L, and the specimen was incubated for 30 min at 37°C with periodic agitation before measurement of surface activity.

Measurements of IL-1 β , LF, and MPO. IL-1 β was measured using an immunoassay (Quantikine, Research and

Diagnostics Systems Inc., Minneapolis, MN). RIA for MPO and LF were performed as described (25).

Presentation of results. The concentrations of PC, SPC, SP-A, MPO, LF, IL-1 β , and nonsedimentable protein in ELF were calculated using urea as a marker of the extracellular space (26). Urea was quantified in serum (recovered within 24 h from the AS) and in AS using an enzymatic method (19). The concentration of PC was calculated as follows: $[PC]_{ELF} = [PC]_{AS} \times \text{urea}_{\text{serum}} / \text{urea}_{AS}$.

The concentrations of surfactant components, MPO, LF, and IL-1 β are shown for specimens collected 1 to 2 d before and 1 to 3 d after the onset of DEX or PL; surface activities of specimens collected 1 to 2 d before and 2 to 5 d after the onset of DEX or PL are also given. For measurements of surface activity and inhibition of surface activity by proteins, specimens from d 2 to 5 were combined to have enough material for testing. Twenty-eight specimens from a total of 14 infants were studied for minimum surface tension of the phospholipid complex, and 20 specimens from 10 infants were studied for inhibition of surface activity. Because data are missing owing to extubation of infants, results of specimens collected more than 5 d after the onset of DEX or PL treatment are not presented.

Statistics. The results are expressed as numbers of infants, means $\pm$ SD or SEM, or medians and quartiles. Two-sided *t* test, Mann-Whitney U test, analysis of variance, Wilcoxon signed rank-test, and χ^2 -test with continuity correction were used. A *p* value of less than 0.05 was considered significant.

RESULTS

Clinical outcome. Some clinical characteristics of the 23 enrolled infants are presented in Table 1. The treatment was started at the postnatal age of 15 d in the DEX group and 14 d in the PL group. There was no significant difference in the ventilatory settings between the groups at entry (Table 2). Three days after entry, DEX-treated infants required significantly less ventilatory support and supplemental oxygen than at entry. Three infants died, one infant in the DEX group (at the age of 125 d) and two in the PL group (at the ages of 22 and 28 d). During the 1-wk treatment period, six (55%) of the infants in the DEX group and two (17%) of the infants in the PL group were weaned from the ventilator (*p* = 0.14), but four of the six DEX-treated infants required later reintubation

Table 1. Characteristics of infants [number of infants or mean (SD)]*

	DEX group (n=11)	PL group (n=12)
Birth weight (g)	873 (223)	803 (110)
Gestational age (wk)	26.8 (1.7)	26.0 (1.5)
Males [n (%)]	8 (73%)	3 (25%)
Postnatal age at start of trial medication (d)	15 (3)	14 (3)

* All comparisons between DEX and PL group were NS.

Table 2. Ventilatory parameters at entry and during treatment period [number of infants or mean (SD)]*

	DEX group (n=11)	PL group (n=12)
At entry		
Rate of ventilation	31 (18)	33 (15)
Peak inspiratory pressure (cm H ₂ O)	20 (3)	20 (3)
Mean airway pressure (cm H ₂ O)	8.3 (2.2)	8.1 (2.2)
Fio ₂ †	0.43 (0.20)	0.42 (0.19)
Fio ₂ $\times$ MAP	4.1 (3.0)	3.5 (1.5)
After 3 d		
Ventilator dependent	9	10
Rate of ventilation	26 (16)	32 (19)
Peak inspiratory pressure (cm H ₂ O)	19 (3)	20 (2)
Mean airway pressure (cm H ₂ O)	6.9 (2.3)	7.8 (3.0)
Fio ₂	0.32 (0.11)‡	0.39 (0.23)
Fio ₂ $\times$ MAP	2.6 (1.6)‡	3.6 (3.0)

* MAP, mean airway pressure; Fio₂ fractional inspiratory oxygen. Conversion: 10 cm H₂O = 1 kPa.

† Mean Fio₂ requirement during the 24 h before start of trial medication (Fio₂ recorded 24, 18, 12, and 6 h before entry).

‡ *p* < 0.05, comparison of ventilatory parameters at entry and after 3 d. All comparisons between DEX and PL group were NS.

and ventilatory support. Median duration of ventilatory support was similar in both groups (DEX 24.0 d, PL 26.0 d; *p* = 0.78). The survival rate at 28 d without ventilatory support or CLD did not differ between the groups [survival without ventilatory support: DEX 5/11 (45%), PL 2/12 (17%), *p* = 0.30; survival without CLD: DEX 1/11 (9%), PL 0/12 (0%), *p* = 0.96].

Nonsedimentable protein. Protein concentrations in ELF at entry were similar in each group ([protein]_{ELF}: DEX 13.6 g/L, PL 9.2 g/L, *p* = 0.48; Fig. 1). After 3 d, the concentrations of protein were significantly lower in the DEX-treated than in the PL-treated infants ([protein]_{ELF}: DEX 2.9 g/L, PL 5.7 g/L, *p* = 0.027). Concentrations of protein in ELF tended to be lower 1 to 2 d after the onset of DEX or PL (data not shown) and were lower after 3 d of treatment in the eight infants weaned from the ventilator during the 1-wk treatment period compared with the 15 infants who were not weaned ([protein]_{ELF}: weaned 1.5 g/L, ventilator dependent 4.0 g/L, *p* = 0.024). The

Figure 1. Total nonsedimentable protein in ELF (g/L). AS were collected 1 or 2 d before and 1 to 3 d after the onset of DEX or PL (mean and SEM). Number of specimens before entry: DEX, *n* = 8; PL, *n* = 8; after 1 d: DEX, *n* = 10; PL, *n* = 7; after 2 d: DEX, *n* = 9; PL, *n* = 10; and after 3 d: DEX, *n* = 8; PL, *n* = 10. *, *p* < 0.05 compared with PL group.

molecular weight distribution of protein in AS was analyzed using gel electrophoresis. There were 18 specimens from six DEX-treated infants and 27 specimens from six PL-treated infants. According to this analysis, the composition of AS protein remained similar in each group during the treatment period. The molecular weight distribution of AS protein was similar to that of serum protein (data not shown). The ratio $[\text{protein}]_{\text{AS}}/[\text{protein}]_{\text{serum}}$ did not differ between the groups before the treatment was started [DEX 4.9 ($n = 3$); PL 5.5 ($n = 5$) ($\text{g} \times \text{L}^{-1}/(\text{g} \times \text{mL}^{-1})$; $p = 0.73$], but it was lower after 2 to 5 d in the DEX-treated than in PL-treated infants [DEX 1.0 ($n = 4$); PL 6.7 ($n = 5$) ($\text{g} \times \text{L}^{-1}/(\text{g} \times \text{mL}^{-1})$; $p = 0.01$].

Surfactant components, surfactant inhibitors, and surface activity of sedimentable surfactant complex. Concentrations of SP-A and PC in ELF before entry into the study and 1 to 3 d after the onset of treatment with DEX or PL are presented in Table 3. There was a wide variation in the concentrations of all surfactant components during the 1-wk treatment period. DEX treatment had no significant effect on concentrations of PC in ELF (Table 3) or on concentrations of SPC in ELF (data not shown). There was no significant difference between the groups in the SP-A concentrations at entry (Table 3). In the PL-treated infants, SP-A concentrations remained similar during the treatment period, whereas in the DEX-treated infants a transient increase in SP-A levels was evident after 1 d (Table 3). Thereafter, the SP-A levels of DEX-treated infants decreased and reached the pretreatment levels (Table 3). During the 3 d after the onset of DEX or PL, concentrations of SP-A, PC, and SPC did not differ significantly between the infants who were weaned from the ventilator during the treatment period compared with those who were not (data not shown).

DEX had no detectable effect on the ratio of SPC/PC. This ratio was similar in both groups at entry (DEX 0.39 mol/mol and PL 0.48 mol/mol; $p = 0.21$) and during the treatment period (after 1 d, DEX 0.46 mol/mol and PL 0.46 mol/mol, $p = 0.93$; after 2 d, DEX 0.41 mol/mol and PL 0.48 mol/mol, $p = 0.46$; and after 3 d, DEX 0.41 mol/mol and PL 0.47 mol/mol, $p = 0.23$). Ratios of SP-A/SPC and SP-A/PC before entry into the study and 1 to 3 d after the onset of the treatment are presented in

Figure 2. At entry, SP-A/SPC and SP-A/PC ratios did not differ between the DEX and PL groups (SP-A/SPC: DEX 62.0 g/mol, PL 47.5 g/mol, $p = 0.55$; SP-A/PC: DEX 29.4 g/mol, PL 19.9 g/mol, $p = 0.28$). One day after the onset of DEX, a short-lived increase was noticed in SP-A/SPC and SP-A/PC ratios (SP-A/SPC ratio: DEX 96.9 g/mol, PL 40.5 g/mol, $p = 0.037$; SP-A/PC: DEX 35.9 g/mol, PL 16.1 g/mol, $p = 0.043$). Thereafter, both ratios of DEX-treated infants decreased and reached the levels of PL-treated infants. Ratios of SP-A/PC, SP-A/SPC, and SPC/PC during the 3 d after the onset of DEX or PL were similar in those infants who were weaned from the ventilator during the treatment period and those who were not (data not shown).

Surface activity of the sedimentable lipid-protein complex of AS, before entry and after 2 to 5 d, is presented in Table 4. Minimum surface tension of the sedimentable surfactant complex did not differ significantly between the groups before entry into the study ($p = 0.15$) or after 2 to 5 d of treatment ($p = 0.55$). There was no difference in the surface adsorption rates (data not shown).

The inhibitory effect of the nonsedimentable protein on surface activity of human surfactant was measured in the AS collected before entry into the study and after 2 to 5 d (Table 4). Before entry, the minimum surface tension of surfactant in the presence of the nonsedimentable protein was similar in both groups ($p = 0.69$). After 2 to 5 d at a constant concentration, the nonsedimentable protein in AS of DEX-treated infants had a lower inhibitory effect on surface activity of human surfactant *in vitro* than protein in AS of PL-treated infants ($p = 0.047$). These surfactant inhibitors did not affect the surface adsorption rates (data not shown). Because too few specimens were available for the study, pulmonary outcome and surface tension measurements could not be correlated.

The impact of the total nonsedimentable protein concentration (4 mg protein/mL and 8 mg protein/mL) and the PC concentration in human surfactant (1.8 mmol/L and 7.2 mmol/L) on surface activity were further studied *in vitro*. The inhibitory effect of the nonsedimentable protein fraction (4 mg protein/mL) on surfactant function disappeared when the concentration of PC in human surfactant was increased from 1.8 mmol/L to 7.2 mmol/L (minimum sur-

Table 3. SP-A and PC in ELF*

	DEX group				PL group			
	Before	After			Before	After		
		1 d	2 d	3 d		1 d	2 d	3 d
[SP-A] _{ELF} (mg/L)								
Median	51	240	133	63†	118	121	95	83
Quartile	27–133	151–365	41–210	24–147	31–144	66–237	66–135	66–144
No. AS	$n = 6$	$n = 8$	$n = 7$	$n = 8$	$n = 7$	$n = 6$	$n = 9$	$n = 9$
[PC] _{ELF} (mmol/L)								
Median	3.1	6.1	5.9	3.8	7.6	3.7	6.5	6.6
Quartile	2.4–4.1	2.3–10.7	3.7–7.5	2.2–5.2	4.5–14.0	3.4–14.7	3.8–10.1	4.0–8.3
No. AS	$n = 6$	$n = 8$	$n = 7$	$n = 8$	$n = 8$	$n = 7$	$n = 8$	$n = 8$

* Specimens were collected 1 to 2 d before and 1 to 3 d after onset of DEX or PL treatment.

† $p < 0.05$ compared with specimens collected 1 d after onset of DEX treatment. All other comparisons were NS.

Figure 2. Ratio of SP-A to PC (g/mol) (upper panel) and ratio of SP-A to SPC (g/mol) (lower panel) in AS. AS were collected 1 or 2 d before and 1 to 3 d after the onset of DEX or PL (mean and SEM). Number of specimens for SP-A/PC before entry: DEX, $n = 7$; PL, $n = 9$; after 1 d: DEX, $n = 9$; PL, $n = 8$; after 2 d: DEX, $n = 7$; PL, $n = 9$; and after 3 d: DEX, $n = 8$; PL, $n = 9$. Number of specimens for SP-A/SPC before entry: DEX, $n = 4$; PL, $n = 6$; after 1 d: DEX, $n = 8$; PL, $n = 7$; after 2 d: DEX, $n = 6$; PL, $n = 8$; and after 3 d: DEX, $n = 6$; PL, $n = 7$. *, $p < 0.05$ compared with PL group; †, $p < 0.05$ compared with d-1 value in DEX group.

face tension of surfactant: 2 mN/m; minimum surface tension of surfactant + inhibitor: 3 mN/m; $p = 0.46$, $n = 5$). However, a higher concentration of nonsedimentable fraction (8 mg protein/mL) significantly increased the minimum surface tension of human surfactant, containing 7.2 mmol/L PC (minimum surface tension of surfactant: 2 mN/m; minimum surface tension of surfactant + inhibitor: 9 mN/m; $p = 0.02$, $n = 4$); the protein fraction from DEX-treated infants tended to be less inhibitory than the

fraction from PL-treated infants (after 2 to 5 d DEX, 3 and 8 mN/m; after 2 to 5 d PL, 9 and 15 mN/m).

Inflammatory mediators: IL-1 β , MPO, and LF. At entry and during the treatment period, MPO and LF concentrations in ELF did not differ significantly in DEX- or PL-treated infants (Table 5). IL-1 β concentrations were also similar in both groups at entry and during the treatment period (Table 5).

DISCUSSION

According to current evidence, DEX treatment of preterm ventilator-dependent infants with threatened CLD decreases the requirements of ventilatory support (3, 5, 7, 8, 16, 18) and improves pulmonary mechanics (4–6). DEX treatment has decreased the concentrations of albumin in lung effluent (5, 15, 16), suggesting decreased endothelial and epithelial permeability. Increased epithelial permeability has been detected in preterm infants with respiratory distress syndrome, and this abnormal permeability persists in infants subsequently developing CLD (27). In the present study, DEX treatment decreased the concentrations of nonsedimentable protein in ELF and also decreased the ratio of protein in AS to protein in serum. The nonsedimentable protein fraction of AS disturbed the ability of the pulmonary surfactant to reach very low surface tension *in vitro*. The inhibition of surfactant function was dependent on both the inhibitor and surfactant concentrations. The inhibition of surface activity was detectable when the concentration of surfactant PC and nonsedimentable protein were within ranges that were evident in ELF of most infants (Fig. 1, Table 3). The minimum surface tensions obtained in the present study were high. However, these patients were critically ill, small, preterm infants who may have had abnormal surfactant function. We measured lower minimum surface tensions when the humidity of ambient air was decreased. Because the humidity of alveolar air is 100%, we chose to report the measurements close to 100% humidity. In a few cases when sufficient quantities of AS were available, the surface tension was confirmed using a modified Wilhelmy balance (28).

In addition to decreasing concentration of protein in ELF, DEX treatment weakened the properties of the nonsedimentable protein as an inhibitor of surfactant function. The cause of the DEX-induced decrease in surfactant inhibitor activity is unknown. Less than 10% of phospholipid or SP-A was recovered in the nonsedimentable fraction of AS, regardless of the treatment. There was no detectable difference between the DEX and PL groups in the molecular weight distribution of the proteins from the nonsedimentable fraction. The molecular weight distribution of the protein in AS was similar to that of serum protein. However, the present method of gel electrophoresis does not detect small differences in protein composition that may account for the observed difference in the inhibitor potency.

Table 4. Surface activity of sedimentable lipid-protein complex in AS and inhibitory effect of nonsedimentable protein on surface activity of human surfactant [mean (SD) or number of specimen]*

	DEX group	PL group
Minimum surface tension of lipid-protein complex (mN/m)	$n = 7$	$n = 7$
Before	19.8 (1.7)	16.3 (5.7)
After 2 to 5 d	17.6 (2.2)	15.8 (7.6)
Minimum surface tension of human surfactant in presence of nonsedimentable protein (mN/m)†	$n = 5$	$n = 5$
Before	16.2 (5.9)	17.4 (1.6)
After 2 to 5 d	12.7 (4.6)	17.7 (1.4)‡

* Specimens were collected 1 to 2 d before and 2 to 5 d after onset of DEX or PL treatment.

† The final concentration of protein in the nonsedimentable fraction of AS was 4 g/L, and the final concentration of surfactant PC was 1.8 mmol/L. The minimum surface tension of human surfactant without inhibitor was 3.9 (0.7) mN/m.

‡ $p < 0.05$ compared with DEX group.

Table 5. Inflammatory parameters in ELF*

	DEX group				PL group			
	Before	After			Before	After		
		1 d	2 d	3 d		1 d	2 d	3 d
[LF] _{ELF} (mg/L)								
Median	71	78	99	97	96	113	89	119
Quartile	38–115	46–96	32–219	37–195	64–126	17–208	67–126	38–282
No. AS	<i>n</i> = 7	<i>n</i> = 5	<i>n</i> = 8	<i>n</i> = 8	<i>n</i> = 8	<i>n</i> = 2	<i>n</i> = 9	<i>n</i> = 7
[IL-1 β] _{ELF} (mg/L)								
Median	2.2	2.2	0.7	1.2	2.1	0.8	1.0	1.0
Quartile	0.1–3.0	1.3–14.9	0.1–1.5	0.9–4.6	0.9–12.5	0.5–1.7	0.6–1.5	0.8–1.3
No. AS	<i>n</i> = 5	<i>n</i> = 7	<i>n</i> = 6	<i>n</i> = 7	<i>n</i> = 7	<i>n</i> = 6	<i>n</i> = 9	<i>n</i> = 8
[MPO] _{ELF} (mg/L)								
Median	118	51	54	47	81	28	40	76
Quartile	68–147	29–71	30–121	25–67	52–121	11–45	24–75	27–214
No. AS	<i>n</i> = 7	<i>n</i> = 5	<i>n</i> = 8	<i>n</i> = 8	<i>n</i> = 8	<i>n</i> = 2	<i>n</i> = 9	<i>n</i> = 7

* Specimens were collected 1 to 2 d before and 1 to 3 d after onset of DEX or PL treatment. All comparisons were NS.

The decrease of protein concentration in ELF during DEX treatment could be due to several mechanisms. Glucocorticoid treatment has been shown to decrease microvascular permeability (29), increase the epithelial clearance of macromolecules (30), and decrease neutrophil recruitment (5, 16), resulting in lung edema (5, 16, 29). In ventilated preterm rabbits, prenatal steroid treatment decreased protein leak across capillary endothelium and increased clearance of protein from the bronchoalveolar lavage (30). It has been suggested that the favorable effect of prenatal steroid treatment in ventilated animals is primarily mediated through pulmonary maturation and not through a direct pharmacologic effect (30). Steroids may have a somewhat different mechanism of action in CLD or early CLD from that detected in prenatal treatment. Several studies have failed to show a sustained beneficial effect of DEX in preterm infants when the treatment was discontinued (3, 5, 18). It has been speculated that respiratory failure seen after discontinuation of DEX is related to the return of the inflammatory state (5). The results of the present study are consistent with the possibility that DEX increases the clearance of plasma-derived proteins from ELF or decreases the flux of plasma-derived proteins into the alveolar space.

In the present study, DEX treatment had no detectable effect on the concentrations of PC or SPC in ELF. Prenatal steroid treatment of rabbits has increased phospholipids in alveolar lavage at preterm birth, but after ventilation the size of the alveolar surfactant pool has been similar in both steroid-treated and control rabbits (30–33). Prenatal steroid treatment has not increased the phospholipid concentrations in amniotic fluid or in gastric aspirates of the newborn infants at birth (34). Our results and results of previous animal studies suggest that postnatal glucocorticoid treatment of ventilator-dependent preterm infants has no clearcut effect on the concentrations of alveolar phospholipids.

One day after the onset of DEX, the SP-A/SPC ratios and the SP-A/PC ratios were significantly higher compared with the corresponding ratios in other specimens

from DEX- and PL-treated infants. Thus, the increase in SP-A/SPC ratio corresponded with a transient increase in the concentration of SP-A in ELF. According to studies using lung explants, glucocorticoid transiently stimulates the synthesis of the mRNA for SP-A (35). SP-A has been shown to be protective against surfactant inhibition caused by specific proteins and amino acids (19, 36) and to delay conversion of the large surfactant aggregates toward the small aggregates that are no longer surface active (37). Moreover, the increase in SP-A may transiently improve the host-defense mechanism of alveolar macrophages (38). The present results do not exclude the possibility that DEX increased the concentrations of surfactant protein-B (39) or surfactant protein-C (40), both of which increase surface activity. In any case, we were unable to detect any qualitative differences between DEX- and PL-treated infants in surface activity of the lipid-protein complex isolated from AS.

DEX treatment started at d 14 had no detectable effect on IL-1 β concentrations in ELF. The MPO and LF levels were also similar in each group. The IL-1 β levels in this study were of similar magnitude to those detected in preterm infants evolving CLD (41, 42). In contrast to our results, Groneck *et al.* (16) found a decrease in the IL-1 β concentrations in tracheobronchial fluid of the preterm ventilator-dependent infants during early DEX treatment starting at 10 d of age but not when DEX treatment was started at 16 d of age. IL-1 β levels tended to be lower in their early treatment group, both before and during the DEX treatment, than in this study (16). High activity of MPO in bronchoalveolar lavage fluid has been associated with lung injury in adults (43). Low levels of LF, an iron-binding protein found in neutrophils and serous cells of airway submucosal glands, are related to immaturity and to development of CLD in preterm infants (44). It has been proposed that high LF levels may reflect protective capacity of the lungs against injury (44). The reason why we did not find any effect of DEX on inflammatory mediators or on LF is unclear. In experimental studies, glucocorticoids suppress inflammation (29) and down-

regulate cytokines (45). Severity of lung injury, timing, dose, and duration of the DEX treatment in this study may explain the failure to detect any effect of DEX on MPO and IL-1 β levels.

In the present study, DEX treatment decreased significantly the concentrations of protein in ELF and decreased the ratio of protein in AS to serum protein, suggesting decreased capillary permeability, increased epithelial clearance of protein, or both. The decrease in the protein concentration of ELF coincided with the decrease in the capacity of this protein to decrease the surface activity. We propose that the acute favorable effect of DEX in CLD and in infants at risk for CLD is in part mediated through improvement of surfactant function: low interfacial surface tension may thus contribute toward clearance of lung edema fluid and increase lung compliance after glucocorticoid treatment.

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