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Surfactant phosphatidylcholine composition during dexamethasone treatment in chronic lung disease

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Abstract

Objectives **– To determine whether dexamethasone 'matures' the phosphatidylcholine (PC) composition of broncheoalveolar fluid in infants at high risk of neonatal chronic lung disease (CLD), either by increasing the proportion of dipalmitoylphosphatidylcholine (DPPC), expressed as a percentage of total PC (%DPPC), or by increasing the ratio of DPPC to palmitoyloleoylphosphatidylcholine (DPPC:POPC ratio).**

Design **– Double blind, placebo controlled.**

Setting and patients **– Sixteen infants <32 weeks' gestation, <1250 g birth weight who were dependent on mechanical ventilation and requiring a fractional inspired oxygen of >0·30 at 12 days of chronological age.**

Intervention **– Randomisation to receive a two week reducing course of dexamethasone base at an initial dose of 0·2 mg/kg three times a day, or equivalent volumes of normal saline, starting at 14 days. Eight infants were randomised into each group. Broncheoalveolar lavage was performed serially throughout the study period or until extubation. PC composition of the fluid was analysed by high performance liquid chromatography.**

Outcome measures **– The %DPPC and the DPPC:POPC ratios were calculated for individual infants for days −1 and 0 combined, days 1 and 3 combined, and days 5 and 7 combined. Analysis of covariance was used to analyse the results.**

Results **– The DPPC:POPC ratio was significantly *less* in the treated group than the placebo group on days 1 and 3, and not greater as the hypothesis stated. Three out of five infants treated with dexamethasone and for whom data were available showed a substantial rise in DPPC:POPC ratio on days 5/7, compared with the placebo group, but overall these changes were not statistically significant.**

Conclusions **– The data do not support the hypothesis that dexamethasone's action in producing a clinical improvement within the first 72 hours of treatment for neonatal CLD is by the 'maturation' of pulmonary surfactant PC.**

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With the emphasis in neonatal intensive care moving towards concerns about morbidity, rather than just mortality, the problem of neonatal chronic lung disease (CLD) is increasing in importance. Neither treatment nor efforts aimed at the prevention of CLD have proved to be completely effective. One of the few treatments which has been shown to work, albeit probably only in the short term, is the use of the corticosteroid dexamethasone.[1–4] Little is known about dexamethasone's mechanism of action in the treatment of CLD, and its use is fraught with concerns over side effects.[5] Despite this, there is increasing use of dexamethasone earlier in the course of respiratory distress syndrome and CLD,[6] and a multicentre trial is currently being planned to study the effects of dexamethasone within 72 hours of life.[5]

There is a lag of between 24 and 72 hours from the onset of administration of dexamethasone and the beginning of both clinical and physiological improvement in infants with CLD.[1–4] Any mechanism proposed to explain the action of dexamethasone in CLD must account both for this delay, and also account for the physiological changes themselves, namely improvements in pulmonary compliance and resistance.[7 8] One mechanism that would fulfil both of these criteria is the induction of enzyme systems responsible for the synthesis of pulmonary surfactant. It has been shown conclusively that antenatal steroids will reduce the incidence of respiratory distress syndrome,[9] and that this must be due, at least in part, to the improved synthesis and/or secretion of pulmonary surfactant in the infant.

The saturated phosphatidylcholine (PC) species dipalmitoylphosphatidylcholine (DPPC) is the major surface active component, in vivo, of pulmonary surfactant. We have shown previously that infants who developed respiratory distress syndrome had a lower percentage of DPPC in the PC content of tracheal aspirates taken at birth, and a lower ratio of DPPC to the more unsaturated PC species palmitoyloleoylphosphatidylcholine (POPC) than infants who did not develop respiratory distress syndrome.[10] This difference appeared to be independent of gestational age. We postulated that the higher percentage of DPPC and the higher ratio of DPPC to POPC (the DPPC:POPC ratio) in the infants who did not develop respiratory distress syndrome represented a 'maturation' of the PC composition of pulmonary surfactant, producing a surfactant which was more effective in reducing surface tension and was at least to some extent the reason why

Table 1 Demographic data, values are medians (interquartile range)

	Birth weight (g)	*Gestational age (weeks)*	*Age at entry (days)*	*Male:female (ratio)*
Treated (n=8)	830 (740–995)	26·5 (24–27)	16 (16–19)	4:4
Placebo (n=8)	770 (745–825)	26·0 (24–26)	16 (15–16)	5:3

these infants did not develop respiratory distress syndrome. This current study was carried out to test the hypothesis that dexamethasone acts in CLD by increasing the percentage of DPPC or by increasing the DPPC:POPC ratio of the infants' pulmonary surfactant, that is, that it acts by 'maturing' the PC composition of pulmonary surfactant.

Patients and methods

Infants <32 weeks' gestation, <1250 g birth weight, and who were still dependent on mechanical ventilation and on a fractional inspired oxygen >0·30 at 12 days of age, were randomised to receive dexamethasone, or placebo, after written informed consent was obtained from the parents. The randomisation was blinded to all participants apart from the pharmacist. The dexamethasone regimen was 0·2 mg/kg/dose of dexamethasone base three times daily for four days, then twice daily for three days, once daily for three days, and then once daily for alternate days to complete 14 days in total. The volume of each dose was made up to 1 ml, and the placebo doses consisted of 1 ml normal saline. The doses were given intravenously if a line was in situ and via the nasogastric tube if not, as it has been demonstrated that enteral dexamethasone is both well absorbed and efficacious in the treatment of CLD.[11] Treatment was started at 14 days of age, or as soon after this point as thought clinically safe by the clinician in charge. Infants could be withdrawn from the study and treated outside of the trial if it was thought to be clinically indicated. The policy of the unit at the time of the study was to use dexamethasone to treat infants with CLD at 28 days of life, if clinically indicated.

The study period extended from two days before starting treatment (day −1), through to the last day of treatment (day 14). Blind broncheoalveolar lavage was performed on days −1, 0, 1, 3, 5, 7, 10, and 14, or until extubation, at times when routine endotracheal tube toilet was considered necessary by the nursing staff. Two French gauge (5 FG) end hole endotracheal tube suction catheters (Vygon) were flushed through with normal saline, and each were attached to a syringe containing 1 ml/kg normal saline. If the ventilator rate was less than 30 breaths/minute, it was adjusted to this level, and the fractional inspired oxygen of the infant was increased to 0·10 higher than the maintenance level, just before the procedure. Infants were monitored by a cardiorater and with either pulse oximetry, or by a transcutaneous oxygen tension electrode. Suction was performed at 1·07 kPa (8 mm Hg). 'Dry' suction of the endotracheal tube only was performed initially. The infants were then positioned supine with their heads turned towards the left. The infants were disconnected from the ventilator, and the first of the preloaded 5 FG catheters introduced into the endotracheal tube, until it was wedged. The saline was then flushed through the catheter, and the ventilator reconnected, with the catheter left in situ. The saline was allowed to dwell for 20 seconds before the ventilator was disconnected again and the saline suctioned out via a modified sputum trap (Unoplast). The catheter was then withdrawn and the ventilator reconnected. The infants were allowed to recover for two minutes, and then the procedure was repeated using the second preloaded syringe and the same sputum trap. Once the lavage was complete, ventilator settings were returned to their original levels. The broncheoalveolar lavage fluid was placed immediately onto ice until centrifuged at 1000 *g* for 10 minutes, at 4°C, to remove cells and cellular debris. The supernatants were aliquoted and stored at −70°C, until analysis.

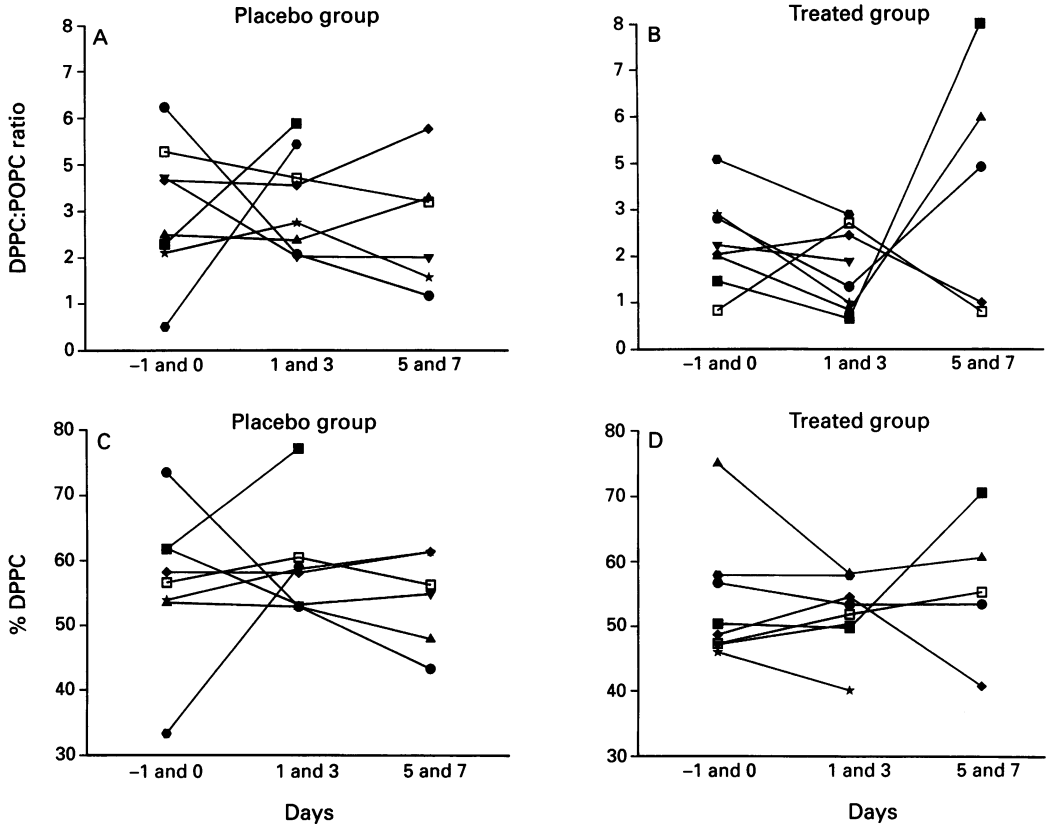
Total lipids were extracted with chloroform:methanol, as described previously.[12] Individual molecular species of PC were resolved by isocratic reverse phase high performance liquid chromatography.[13] The proportion of DPPC was expressed as a percentage of total PC (%DPPC) and the DPPC:POPC ratio calculated for each lavage sample.

For each infant both the mean %DPPC and DPPC:POPC ratio was calculated for days −1 and 0 combined and for days 1 and 3 combined, as this was the period over which a clinical improvement was expected to occur. Where possible, values were also calculated for days 5/7. Analysis of covariance and the Mann-Whitney U test, using the Statgraphic software package, were used to analyse the resulting data.

Approval for the study was granted by local ethics committees.

Results

Nineteen infants fulfilled the entry criteria during the study period. Consent was declined for one, and the clinician in charge considered that two were too ill to be randomised. Sixteen infants were recruited; eight received dexamethasone and eight placebo. The demographic data are shown in table 1. There were no significant differences between the groups for gestational age, birth weight, or the age at which treatment started. One infant who was randomised to receive dexamethasone was withdrawn from the study on day 6 and restarted on dexamethasone because of a failure to respond. Four infants in the placebo group were withdrawn from the study: two during day 4, one during day 5, and one during day 7 (a total of four out of eight withdrawn) because of a deteriorating clinical condition. All four subsequently received unblinded dexamethasone. Two infants from the dexamethasone group were successfully extubated during day 3. Analysis of the fluid from the



DPPC:POPC ratios for individual infants in (A) placebo and (B) treated groups and %DPPC for individual infants in (C) placebo and (D) treated groups.

broncheoalveolar lavage performed on one infant on day 5 was unsuccessful, so no data were available.

The results for the individual infants are shown graphically in the figure. Analysis of covariance was carried out, with treatment with dexamethasone acting as the dummy variable. The results are shown in table 2. These show that the DPPC:POPC ratio for days 1 and 3 is significantly lower in the group treated with dexamethasone compared with the placebo group. There was a similar but not significant difference for %DPPC. Neither of these findings remained apparent for days 5 and 7, but in three out of the five infants for whom data were available, there was a marked increase in the DPPC:POPC ratio for days 5/7.

Discussion

These results do not support the hypothesis that dexamethasone acts in neonatal CLD by maturing pulmonary surfactant PC composition during the initial 72 hour period when the clinical response is observed. The significance of the lower DPPC:POPC ratio in the treatment group for days 1 and 3 is not clear. The apparent maturation seen in three infants treated with dexamethasone for days 5/7 is interesting, but with such small numbers it is not possible to draw any firm conclusions. However, it has been demonstrated that in preterm rabbits, antenatal steroids given for 48 hours before delivery did not increase the alveolar surfactant pool size, but did produce changes in pulmonary protein leak which would have led to the clearance of protein and fluid from the alveoli and airways.[14] It was found that despite the lack of increased pulmonary surfactant, an improvement in lung compliance occurred, and it was suggested that this improvement was the result of a corticosteroid 'induction' of the mechanisms responsible for the clearance of fetal lung fluid. In our own study, we found that the clinical improvements seen with dexamethasone coincided with a diuresis, which may be the result of increased pulmonary fluid resorption (unpublished data). We speculate that the postnatal effects of dexamethasone in CLD may mimic the antenatal effects seen by Jobe's group[14] with clearance of lung fluid being followed by an improvement in surfactant. Larger numbers of infants would have to be studied to confirm or refute this. What remains certain, however, is that the exact mechanism of dexamethasone's action in CLD is as yet undetermined.

Table 2 Results of analysis of covariance for the dummy variable (treatment with dexamethasone)

	Regression coefficient	*95% Confidence interval*
DPPC:POPC ratio		
Days 1 and 3	−2·0	−3·3 to −0·6
Days 5 and 7	1·0	−3·2 to 5·2
%DPPC		
Days 1 and 3	−6·3	−15·6 to 3·0
Days 5 and 7	2·8	−10·5 to 16·1

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