Turnover of exogenous artificial surfactant

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Abstract

The turnover of the artificial surfactant Exosurf after its administration to infants with respiratory distress syndrome was studied. High performance liquid chromatography was used to compare the phosphatidylcholine (PC) composition of serial endotracheal tube secretions from three groups of infants. There were 22 infants who received two doses of Exosurf in 24 hours (group 1), 10 infants who received four doses in 36 hours (group 2), and 41 control infants who did not receive Exosurf. Two parameters were studied: (i) dipalmitoylphosphatidylcholine (DPPC), which is present in both Exosurf and endogenous surfactant, expressed as a percentage of total PC (% DPPC) and (ii) the ratio of DPPC to the entirely endogenous palmitoyloleoylphosphatidylcholine (DPPC:POPC ratio).

The administration of Exosurf produced changes in endotracheal tube aspirate PC composition that were detectable for over one week. Four doses of Exosurf in 36 hours prolonged the persistence of these changes compared with two doses in 24 hours, but the numbers of infants were small, and should not be overinterpreted. We conclude that after giving two doses of Exosurf, further doses might best be delayed until after two days, and that further clinical evaluation of dosage regimens is required.

Surfactant replacement is rapidly becoming an accepted part of the management of neonatal respiratory distress syndrome, and there are several multicentre clinical trials using exogenous surfactants in progress. There is currently little information available as to the turnover of these products in humans, information that would be a useful adjunct in determining dosage protocols.

The component of the endogenous pulmonary surfactant lipoprotein complex mainly responsible for decreasing the surface tension of the surface monolayer formed at the air-liquid interface in the alveolus is the saturated phospholipid dipalmitoylphosphatidylcholine (DPPC). In addition, pulmonary surfactant contains other phosphatidylcholine (PC) species, other phospholipids, and proteins. Exogenous surfactants fall into three main groups: artificial, natural/animal, and supplemented animal products. The artificial surfactants contain DPPC and agents to aid its adsorption to the surface monolayer, whereas the natural surfactants again contain many types of PC, including DPPC, as well as other phospholipids and proteins.

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Accepted 1 November 1991

Hallman *et al*[1] and Wilkinson *et al*[2] have both used the presence of the phospholipid phosphatidylglycerol (PG) in endotracheal tube secretions of infants treated with human and artificial (ALEC) surfactants respectively to study the turnover of exogenous surfactant. The advantage of studing PG is that it is a phospholipid found only in the surfactant of infants who do not develop respiratory distress syndrome, and is not detected in the initial endotracheal tube secretions of premature infants who do develop respiratory distress syndrome.[3] It was present in the human surfactant used by Hallman's group, as this surfactant was harvested from mature amniotic fluid, and it is the second major component of ALEC, after DPPC. Thus its detection in endotracheal tube secretions of babies with respiratory distress syndrome who had been treated with one of these exogenous surfactants, could be taken as evidence of the presence of the exogenous surfactant. Hallman *et al* calculated a mean half life of the PG component of exogenous human surfactant of 30 hours. Wilkinson *et al* could not detect PG in endotracheal tube aspirates after 12 hours. They also found that the lecithin:sphingomyelin ratio of endotracheal tube secretions from these infants was acutely raised, but had returned to pretreatment values within 20 hours. However, Hallman *et al* could detect significant increases in the saturated PC:sphingomyelin ratio in treated infants, compared with control infants, for one week.[1] This has led to the suggestion that synthetic surfactants may be cleared more rapidly than natural surfactants.[4]

We have been participating in two international multicentre trials involving the artificial surfactant Exosurf (Wellcome). Exosurf contains DPPC, and hexadecanol and tyloxapol are added, to enable it to be incorporated into the surface monolayer. It is administered as a suspension in a dose of 5 ml/kg (67·5 mg DPPC/kg) via the endotracheal tube of ventilated infants. Hexadecanol is a naturally occurring alcohol, which is rapidly metabolised to palmitate, some of which could be processed to DPPC, and tyloxapol is a non-ionic surfactant. We have developed a sensitive method for the detection of different PC species in the endotracheal tube aspirates of ventilated infants and from the lungs of animals.[5 6] Two parameters have been studied: the amount of DPPC, expressed as a percentage of the total PC, and the ratio of DPPC to palmitoyloleoylphosphatidylcholine (POPC): the DPPC:POPC ratio. The DPPC recovered represents both exogenous and endogenous DPPC—it is not possible to distinguish between the two. POPC, a mono-

unsaturated species found in relatively high concentrations in premature infants, is purely endogenously derived. The ratio of DPPC to POPC should therefore reflect the ratio of exogenous and endogenous surfactant assuming that the administration of Exosurf has no effect on POPC, an assumption that seems reasonable from studies of fetal lung remodelling of PC.[7] This information can be used to determine the rate of turnover of Exosurf.

The studies of Wilkinson and Hallman used ratios of non-specific parameters (lecithin and saturated PC, respectively) that reflect the components of the pulmonary surfactant that confer surface activity to the surfactant lipoprotein complex, to sphingomyelin. The study presented here demonstrates a novel approach to the study of exogenous surfactant, as both DPPC and POPC are specific constituents of pulmonary surfactant. Our aims were to determine the period of time over which changes in the PC composition of endotracheal tube aspirates of infants who were treated with two or four doses of Exosurf returned to that of a control group, and to determine any differences in this period between the two treatment regimens.

Subjects and methods

The controls were 41 babies admitted to the neonatal unit during the 18 months before the commencement of the studies on Exosurf (the 'Exosurf' and 'Osiris' multicentre trials), at times when the research staff were available. They were all less than 34 weeks' gestation and were ventilated from day 1 for respiratory distress syndrome diagnosed both clinically and by the typical ground glass appearance and air bronchogram on chest radiograph, by an independent observer. It was not possible to have a contemporaneous control group, because to do so would have denied those infants a treatment that preliminary reports had indicated could be beneficial to their outcome.

The treated infants fell into two groups:

GROUP 1

This group comprised 22 sequential infants less than 34 weeks' gestation who were ventilated from day 1 for respiratory distress syndrome (diagnosed as above) and who received two doses (67·5 mg DPPC/kg/dose) of Exosurf in the first 24 hours.

GROUP 2

This group comprised 10 sequential infants less than 35 weeks' gestation, ventilated from day 1 for respiratory distress syndrome (diagnosed as above), and who received four doses (67·5 mg DPPC/kg/dose) of Exosurf in the first 36 hours.

The duration of recruitment of treated infants was 22 months, from June 1989.

Exosurf was administered via the endotracheal tube, according to the Exosurf or Osiris trial protocols. The first dose was given at a mean age of 6 hours (range 1–12 hours), and doses two, three, and four were given at 12 hour intervals. Day 1 was taken as the 24 hours that included the first three doses. Dose 4 was given at 36 hours (that is, on day 2).

Endotracheal tube aspirates were collected at the time of normal endotracheal tube toilet by nursing staff. All treated infants had a predose sample collected, and each infant was sampled on each day, if they remained intubated. Approximately 0·25–0·5 ml of normal saline were introduced into the endotracheal tube, and were left to dwell for approximately 10 seconds. Deep endotracheal suction was then carried out and suction traps and catheters saved. For the treated group, aspirates were saved before the first two doses of Exosurf, and then three aspirates a day were collected and pooled to form a single daily specimen. For the control babies, three aspirates had been collected each day from approximately 4 hours of age, and pooled to form single daily specimens. Traps and catheters were washed out with 1·6 ml normal saline, and the washings centrifuged at 1000 *g* for 10 minutes to remove cellular debris. The supernatants were stored at −20°C until analysis.

Total lipids were extracted with chloroform: methanol, after PC 14:0/14:0 had been added as an internal standard, and were dried under nitrogen before being dissolved in chloroform. The PC fraction was obtained by selective elution from 100 mg Bondelut NH2 columns (Jones Chromatography) with chloroform: methanol (3:2, volume:volume). Individual PC species were resolved by isocratic reverse phase high performance liquid chromatography (HPLC). A 25 cm Apex II ODS column was used, with a mobile phase of 40 mM choline chloride in 92·5% methanol, 7·5% water at 1 ml/minute. Eluted peaks were quantified by postcolumn fluorescence. The methanol HPLC stream was mixed with an aqueous stream of 1,6-diphenyl-1,3,5-hexatriene at 3 ml/minute, and the resultant fluorescent peaks detected by excitation at 340 nm and emission at 460 nm. The lower limit of detection for each molecular species was 200 pmol.[8–10]

The arterial:alveolar (a:A) ratio, as calculated from the alveolar gas equation, is an index of the severity of respiratory distress syndrome with values falling as severity increases. It was calculated at the time of the initial aspirate for all infants.

Comparison between groups was with the Mann-Whitney U test for the PC analysis data, and both the pooled Student's *t* test and the Mann-Whitney U test for the demographic and a:A ratio data. The χ^2 test was used to compare the proportion of 'successful' samples in each group. The Statgraphics software package was used throughout.

Ethical approval was given by the Southampton and South West Hampshire joint ethical subcommittee for both the Exosurf and Osiris trials, and for the collection of endotracheal tube aspirates.

Results

The initial a:A ratios, birth weight, and gestational age for the two treatment groups and the

controls are shown in table 1. There were no significant differences for any variable between any of the groups. One control infant and one infant from group 1 died during the course of the study.

Table 2 shows the number of infants on each day who had detectable PC in their endotracheal tube aspirates and the number of babies intubated on each day.

Figure 1 shows a typical chromatograph obtained from an infant during this study. She had received two doses of Exosurf three days previously. The area under each peak is proportional to the concentration of that particular molecular species, but it is not possible to calculate absolute concentrations as accurate estimation of the volume of endotracheal tube secretions recovered and the degree of their dilution by the normal saline lavage is not feasible. Results for a particular PC molecular species are therefore expressed as the percentage that species contributes to the total amount of PC recovered.

Table 1 Demographic data and initial a:A ratios. Results are median (range)

	Controls (n=41)	*Group 1 (2 doses) (n=22)*	*Group 2 (4 doses) (n=10)*
Gestation (weeks)	29·0 (25–33)	27·5 (24–33)	28·5 (24–34)
Birth weight (g)	1160 (690–2240)	1095 (700–1840)	1090 (760–2450)
a:A ratio	0·13 (0·07–0·46)	0·15 (0·06–0·44)	0·10 (0·04–0·19)

Table 2 Numbers of infants in each group

	Controls		*Group 1*		*Group 2*	
	Intubated	*Samples*	*Intubated*	*Samples*	*Intubated*	*Samples*
Before first dose/day 1	41	26	22	7	10	4
Before second dose	—	—	22	13	10	8
Day 2	38	21	21	17	10	9
Day 3	37	18	21	20	10	8
Day 4	34	16	20	16	10	8
Day 5	33	18	18	12	8	6
Day 6	27	15	16	12	8	5
Day 7	24	14	14	6	7	5
Day 8	21	10	11	8	6	2
Day 9	19	11	11	6	5	3
Day 10	19	9	11	7	5	4

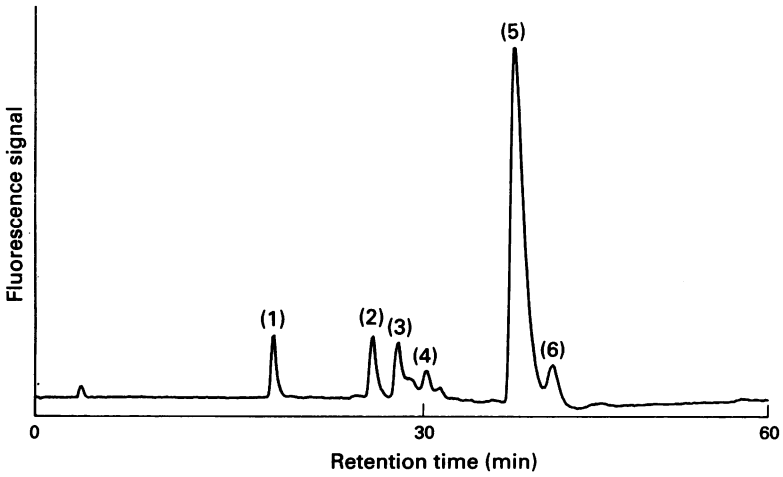

Figure 1 Typical chromatograph three days after administration of two doses of Exosurf. Peaks: (1) Internal standard, PC 14:0/14:0 (dimirystoyl-PC); (2) PC 14:0/16:0 (mirystoylpalmitoyl-PC); (3) PC 16:0/16:1 (palmitoylpalmitoleoyl-PC); (4) PC 16:0/18:2 (palmitoyllinoleoyl-PC; (5) PC 16:0/16:0 (dipalmitoyl-PC); (6) PC 16:0/18:1 (palmitoyloleoyl-PC).

Figure 2 shows the percentage DPPC (%DPPC) in serial endotracheal tube aspirates from an infant who received three doses of Exosurf in the first 24 hours, but who, because of tube blockage, was reintubated with clean endotracheal tubes, three times in the 36 hours after the final dose of surfactant had been given. Despite this repeated change of endotracheal tube, the %DPPC present in the endotracheal tube secretions remained unaffected. Although this evidence is anecdotal, this infant is representative of several infants who required reintubation after completion of Exosurf administration. In addition we also observed infants who were not reintubated and in whom a rapid fall towards control values of both %DPPC and DPPC:POPC ratio occurred.

Figure 3 shows the %DPPC in the endotracheal tube for controls and both treatment groups. The administration of one dose produced a highly significant increase in the %DPPC, compared with the day 1 controls. A second dose caused a further rise, and in many cases the amount of DPPC recovered was so large that it spread during HPLC and swamped the other types of PC completely. These specimens were allocated a %DPPC of 100%, although other PC species were almost certainly present, but in

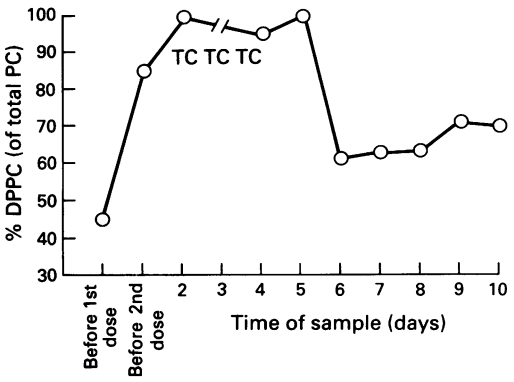

Figure 2 Percentage DPPC and time for an infant who received three doses of Exosurf in 24 hours. TC=endotracheal tube change.

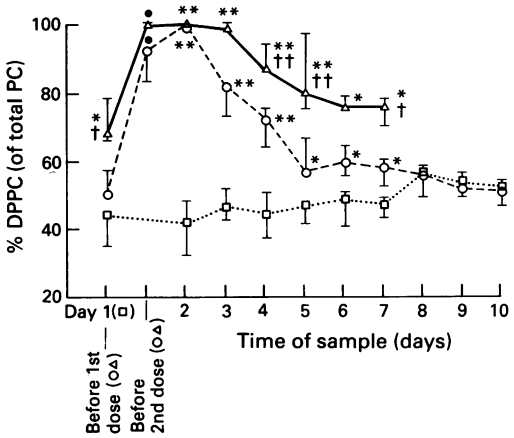

*Figure 3 Percentage DPPC and time for group 1 (circles), group 2 (triangles), and controls (squares). Bars represent lower and/or upper quartiles; ●=p<0·001, compared with day 1 controls; **p=<0·001, compared with same day controls; *=p<0·05, compared with same day controls; ††=p<0·01, compared with same day group 1 infants; †=p<0·05, compared with same day group 1 infants.*

such comparatively small amounts as not to be detectable.

Significant differences, between group 1 and controls for the %DPPC, persisted up to and including day 7. The %DPPC was significantly higher in group 2 specimens taken before the first dose compared with both controls and group 1. However, similar dramatic rises in %DPPC to those seen for group 1 were demonstrated. As infants recovered the number of infants who remained intubated, and from whom samples could therefore be taken, fell (see table 2). Beyond day 7, the numbers in group 2 were very small and so have not been included in this analysis. Significant differences between group 2 and controls persisted for the whole seven days. In addition group 2 infants had a %DPPC that was significantly greater than group 1 infants on days 4, 5, and 10.

Figure 4 compares the DPPC:POPC ratio for both treatment groups and controls. The highest recorded DPPC:POPC ratio was 137. Specimens in which DPPC swamped the other types of PC were allocated a DPPC:POPC ratio of 150. A rise in the DPPC:POPC ratio compared with controls similar to that observed for the %DPPC occurs and, for group 1, is maintained to a significant level until day 4, but with a trend persisting until day 7. For group 2 the ratio remains significantly high compared with controls for six days. Group 2 had a significantly greater DPPC:POPC ratio compared with group 1 on days 4, 5, and 7.

MISSING DATA

As has been previously reported,[1] it is not always possible to detect phospholipids in endotracheal tube aspirates if the volume of aspirate is low. This is a particular problem in the first 24–48 hours. For this reason, not all samples could be successfully analysed. However, there were no significant differences in the proportion of 'successful' samples between the groups after χ^2 analysis of appropriate contingency tables.

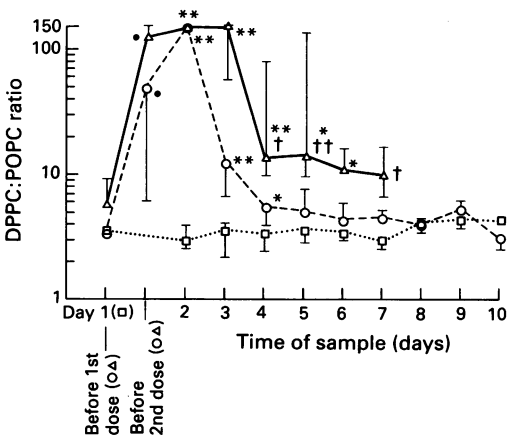

*Figure 4 DPPC:POPC ratio and time for group 1 (circles), group 2 (triangles), and controls (squares). Bars represent lower and/or upper quartiles; ●=p<0·001, compared with day 1 controls; **p=<0·001, compared with same day controls; *=p<0·05, compared with same day controls; ††=p<0·01, compared with same day group 1 infants; †=p<0·05, compared with same day group 1 infants.*

Discussion

When infants were given two doses of Exosurf within the first 24 hours a significant increase in the %DPPC could be detected for the first week of life when compared with controls. The ratio of DPPC:POPC, which reflects the ratio of exogenous to endogenous surfactant, showed a similar trend, with differences significant until day 4. These findings are entirely in keeping with those of Hallman *et al* who, using human surfactant, detected significant increases in the saturated PC:sphingomyelin ratio, compared with control infants, for 1 week.[1] These data contradict the suggestion that artificial surfactants are cleared more rapidly from the lung than natural surfactants.[4]

When four doses of Exosurf were given in 36 hours similar acute changes in both %DPPC and DPPC:POPC ratio were noted. The number of infants who received four doses of Exosurf was small (n=10) and was reduced after five days as babies were extubated. In addition, the initial median %DPPC for group 2 was significantly greater than either the control group, or group 1, though the initial a:A ratio data suggest that the group 2 infants were at least as severely affected as the others, and there were no significant differences between the initial DPPC:POPC ratios. These data should not be overinterpreted, but they do show an interesting trend: the %DPPC was significantly raised over controls throughout the seven days, and the DPPC:POPC ratio was raised significantly until day 6. There was also a persisting difference for both %DPPC and the DPPC: POPC ratio when the two treatment groups were compared. This difference persisted beyond the time that would be expected because of the later administration of the third and fourth doses.

It is possible that analysis of composition of PC from the endotracheal tube might only reflect the PC content of the secretions lining the inside of the endotracheal tube, and that endotracheal tube secretions do not reflect lung fluid itself. However, babies such as the baby whose results are illustrated in fig 2 and who needed reintubation after completion of the surfactant dose did not show the dramatic fall in %DPPC that would be expected if all that was being measured was the content of fluid lining the endotracheal tube. The other compelling piece of evidence is that in most of the aspirates species of PC other than DPPC were detected, indicating that at least part of the sample is derived from the lower respiratory tract.

The results for both treatment groups emphasise that merely replacing the surface active material neither prevents respiratory distress syndrome nor does it cure it. The %DPPC and DPPC:POPC ratio are both very high on days 2, 3, and 4 and exceed values reported for term infants who did not develop respiratory distress syndrome,[5] but all of these infants were being ventilated for the syndrome. However, recently published data do show a reduction in mortality from respiratory distress syndrome.[11–13]

The persistence of the changes in the PC composition could be the result of the exogenous surfactant just inertly lining the alveolar air-

space. However, animal studies suggest that the situation is much more dynamic. Alveolar wash recovered only 19% of radiolabelled PC from exogenous sheep surfactant from preterm ventilated sheep three hours after treatment.[14] Altogether 55% was recovered from lung tissue, suggesting a rapid intake, presumably by type II alveolar cells, after administration. Similar results were obtained three hours after labelled PC in exogenous sheep, calf, and supplemented bovine surfactant was given to 3 three day old rabbits.[15] It is likely that the DPPC from Exosurf is taken up by type II alveolar cells and recycled. Perhaps the most unusual aspect is the persistence of the changes. Studies on 3 day old rabbits showed that labelled exogenous rabbit surfactant was cleared from the lungs at a rate that was almost five times slower than in adult rabbits.[16] Similar age related differences in humans might account for the persistence of PC composition changes.

The number of doses of an exogenous surfactant, and their timing, will have important cost implications. At present the only published data for Exosurf refers to regimens using one or two doses,[11–13] and it is likely that the analysis of data from the Osiris trial in which two and four dose regimens have been compared, will take some time to be completed. Although a regimen of four doses in 36 hours prolonged the period during which changes in the PC composition in the endotracheal tube could be detected to at least six days, large changes were detectable for at least four days when two doses were given in 24 hours. We suggest, therefore, that if more than two doses of Exosurf are to be considered, then delaying the administration of subsequent doses until after day 2 might represent a logical step. Further clinical evaluation of the timing of dosage regimens is required.

We would like to thank Mrs Susan Smith, Mrs Jane Bamber, and the nurses of the neonatal unit in Southampton for their help in collecting the aspirates, and Mrs Smith and Mrs Bamber for their assistance in the collection of clinical data. The project was supported by grants from the Wessex Medical Trust and from Wellcome Foundation Ltd.

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