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Distribution of Exogenous Surfactant in Rabbits with Severe Respiratory Failure: The Effect of Volume

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ABSTRACT. The transient effect of surfactant therapy that is observed in some patients might, at least in part, be explained by a nonhomogeneous distribution. Therefore, we investigated the distribution of a surfactant preparation (Alvofact, 45 g/L) that is used clinically. Rabbits with severe respiratory failure were treated with this surfactant at a dose of 100 mg/kg body weight, and the distribution of surfactant was determined by the use of ^{141}Ce-labeled microspheres that were mixed with the surfactant. Fifteen min after surfactant administration, the rabbits were killed, and the lungs were removed and divided into 200 pieces. The radioactivity per mg lung tissue was determined in each piece. We found that the endotracheal instillation of this surfactant preparation results in a nonhomogeneous distribution. However, a significantly improved distribution was obtained when this dose of surfactant (100 mg/kg body weight) was diluted with normal saline to a concentration of 6.25 g/L. The consequence of the administration of this dose was an intratracheal fluid administration of 16.0 mL/kg body weight. The distribution was also nonhomogeneous after the administration of a small-volume (2.4 mL/kg body weight), low-concentration surfactant preparation (6.25 g/L). We conclude that a surfactant preparation with clinical application is distributed nonhomogeneously in the lungs after endotracheal administration. The distribution can be significantly improved by increasing the fluid volume in which the surfactant is suspended. (*Pediatr Res* 34: 154–158, 1993)

Abbreviations

RDS, respiratory distress syndrome
BW, body weight
PEEP, positive end-expiratory pressure

Since the initial report by Fujiwara *et al.* (1) in 1980, surfactant treatment of newborn infants with neonatal RDS has been studied in clinical trials (2–9). In these studies, the reduction of the severity of respiratory distress, reduction of the incidence of a variety of complications of prematurity, and decreased mortality rates after surfactant treatment have been reported. Besides these favorable results, other studies reported that not all patients respond to the surfactant treatment, or that in some of these newborns the effects are only short-term (10, 11). In addition, the long-term effects of surfactant therapy on mortality and chronic lung disease have not been consistent. The incomplete success of surfactant treatment can be explained by several factors that have been found in experimental studies, including structural immaturity of the lung tissue, protein leak into the alveolar space, and nonhomogeneous distribution of the instilled surfactant (12, 13).

It is currently assumed that the optimal effect will occur when instilled surfactant is distributed homogeneously throughout the lung (14–16). However, little is known about the initial distribution when it is endotracheally instilled in patients. In a previous study, we found that the distribution of exogenous surfactant administered selectively to the left lower lobe of normal rabbits was not homogeneous (17). In that same study, the use of a larger volume of a lower concentration of surfactant improved the distribution in the left lower lobe. So far, the distribution of surfactant over the different lung lobes after endotracheal administration has not been studied in detail.

The aim of the study was to investigate the distribution of a surfactant preparation that is presently used to treat newborn infants with RDS. We studied the distribution of surfactant that was labeled with ^{141}Ce microspheres in rabbits with severe respiratory failure.

MATERIALS AND METHODS

Animals. Three-mo-old Chinchilla rabbits, weighing 2.5 ± 0.3 kg (mean ± SD) were anesthetized with sodium pentobarbital (30 mg/kg BW i.v. Nembutal, Abbott Laboratories, North Chicago, IL) and put into the supine position on a heated mattress. A catheter was introduced into the left carotid artery to measure the arterial blood pressure and to obtain samples for blood gas analysis. After intubation by means of a tracheostomy, the rabbits were paralyzed with pancuronium bromide (Pavulon, 0.1 mg/kg BW) and artificially ventilated (Amsterdam Infant Ventilator MK III, Hoek Loos Co., Schiedam, The Netherlands) with 100% oxygen, with a minute volume of 1.0 L/kg BW at a frequency of 60/min and an inspiration time of 40%. Then the lung-lavage procedure was carried out according to the protocol that we have described previously (18). Briefly, the lungs were lavaged five times at 5-min intervals with 35 mL/kg BW normal saline (38°C). During this procedure, the instillation of normal saline was stopped when the intratracheal pressure reached 3922.4 Pa. The rabbits were allowed to recover from each lavage for a 5-min period. To avoid severe hypoxemia, the level of the PEEP was increased stepwise to 980.6 Pa. Fifteen min after the lung-lavage procedure, the PEEP was decreased to 490.3 Pa.

Surfactant distribution. Five groups of animals were studied

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(Table 1). In all the groups except group A, the rabbits underwent the lung-lavage procedure. All rabbits received bovine surfactant (Alvofact, 45.0 g/L, Boehringer, Ingelheim, Germany). In groups A to D, the surfactant dose was 100 mg/kg BW. The standard concentration of surfactant was diluted with normal saline to a concentration of 12.5 g/L (group C animals) and 6.25 g/L (group D animals), respectively. Consequently, the volumes of the administered surfactant suspensions were 2.4, 8.0, and 16.0 mL/kg BW, respectively. In group E animals, a low-concentration (6.25 g/L), low-volume (2.4 mL/kg BW) dose was given.

To determine the effect of introducing a large fluid volume, we recorded the intratracheal pressure during the administration procedure. We found that the intratracheal pressure increased only 196.1 Pa when a dose of 16.0 mL/kg BW diluted surfactant was administered.

^{141}Ce-labeled microspheres with a diameter of 15×10^{-6} m (Dupont-de Nemours, 's Hertogenbosch, The Netherlands) were mixed with the surfactant by gently stirring. Each animal received approximately 1.5×10^{4} Bq of radioactivity. Fifteen min after the PEEP was decreased to 490.3 Pa, the artificial ventilation was interrupted, and the surfactant was immediately instilled endotracheally through a side lumen of the tube in approximately 45 s. After the instillation, the side lumen of the tube was flushed with 1.5 mL of 0.9% NaCl to remove the residue of the surfactant, and then the artificial ventilation was resumed. During the procedure, the supine position of the rabbits was unaltered.

Processing of lungs. Fifteen min after surfactant instillation, the animals were killed with an overdose of pentobarbital. The lungs were removed, separated into lobes, and frozen on liquid nitrogen. The lobes of the right lung were each cut into 33 pieces, the left upper lobe into 41 pieces, and the left lower lobe into 60 pieces. Each piece of lung tissue was weighed on an analytical balance with a resolution of 0.1 mg (Mettler H35, Mettler-Toledo AG, Zürich, Switzerland). The radioactivity of the ^{141}Ce microspheres was measured. The partition of the endotracheally instilled surfactant over the different lobes of the lungs was determined as follows: the sum of the radioactivity of the pieces from each lobe was expressed as a percentage of the total radioactivity in the lungs. To assess the distribution of the endotracheally instilled surfactant over the 200 different lung pieces, in each piece, the radioactivity was expressed as cpm/mg of lung tissue. Then the mean cpm/mg of all the lung pieces was determined for every rabbit, and in each piece of lung tissue the ratio of the actual cpm/mg and the mean cpm/mg were calculated. In this way, a normalized value for the radioactivity per mg was obtained for every piece of lung tissue. We grouped the normalized values in distribution intervals of 10%. Histograms were obtained by plotting the number of lung pieces in the 10% distribution intervals. Pieces with a normalized value of <0.1 or >2.0 were grouped at the extremes of the distribution intervals. To compare the distribution in the different lobes, we calculated the percentage of pieces with a normalized value between 0.8 and 1.2, because we arbitrarily considered a normalized value between 0.8 and 1.2 as a homogeneous distribution. A higher percentage of lung pieces in this distribution interval represents a more homogeneous distribution of the administered surfactant.

^{99m}Tc scintigraphy. In two rabbits that were subjected to the lung-lavage procedure, we administered 2.4 mL of surfactant/kg body weight at a concentration of 45.0 g/L. Two different labels were mixed through the surfactant: ^{141}Ce-labeled microspheres (Dupont-de Nemours) and ^{99m}Tc-labeled macroaggregated albumin (Amersham, Buckinghamshire, England). The ^{99m}Tc radioactivity in the lungs was measured by scintigraphy while the animals were lying in the supine position. This registration began 15 min after surfactant instillation. A gamma camera was positioned above the thorax of the animal and linked to a computer for acquisition during 2 min in a frame of 64 × 64 pixels (picture elements). Nonspecific background was filtered using a threshold of 10%. The contours of the lungs were drawn automatically on the 10% threshold on the monitor. Similar to the study of Charon *et al.* (19), the lung was divided into four regions: a hilar region, left and right, and a peripheral region of left and right lung. The number of counts in each region was measured. At the end of the experiments, the lungs were excised and processed as described above. The radioactivity of ^{99m}Tc in all lung pieces was measured, and after 5 d of allowing this isotope to decay completely, the radioactivity of the ^{141}Ce microspheres was measured in all lung pieces.

Table 1. *Surfactant dose, concentration, and volume administered in normal and lung-lavaged rabbits*

Group	*n*	Dose (mg/kg BW)	Concentration (g/L)	Volume (mL/kg WB)
A: normal	5	100.00	45.00	2.4
B: lung-lavaged	6	100.00	45.00	2.4
C: lung-lavaged	5	100.00	12.50	8.0
D: lung-lavaged	6	100.00	6.25	16.0
E: lung-lavaged	5	13.75	6.25	2.4

Measurement of blood gases. Blood samples for the determination of arterial P_{O_2}, P_{CO_2}, and pH were obtained from the left carotid artery. Samples were taken after the start of ventilation but before the lung-lavage procedure, after the lung-lavage procedure but before the administration of surfactant, and 15 min after surfactant administration. Samples were analyzed using a ABL 330 blood gas analyzer (Radiometer Co., Copenhagen, Denmark).

Statistical analysis. All data are presented as means ± SEM, unless stated otherwise. Differences between the groups were tested by analysis of variance, and significance was assessed by the Student-Neuman-Keuls multiple-comparison procedure.

RESULTS

Partition of surfactant. We found no significant differences between the percentages of the total ^{141}Ce counts over the different lung lobes in all rabbits independent of surfactant concentration or lung-lavage procedure (Table 2). In all groups, most of the surfactant was found in the left lower and right lower lobe proportional to the weight of these lung lobes.

Distribution of surfactant. The distribution of surfactant in the concentration of 45.0 g/L in normal rabbits and in lung-lavaged rabbits, respectively, was not homogeneous (Fig. 1*A* and *B*). A large number of pieces in the distribution interval <0.2 indicates that a substantial part of the lung receives probably very little surfactant after instillation. Concurrently, we found approximately 35% of all lung pieces in the distribution intervals >2.0, indicating that a significant part of the lung received a large quantity of surfactant. No significant differences were observed between these scores in normal lungs (*A*) and lavaged lungs (*B*), except in the right lower lobe. The distribution of surfactant to the lungs of the animals in group C demonstrated a pattern that is similar to that in groups A and B (Fig. 1*C*), and a slightly higher percentage of lung pieces with a normalized value between 0.8 and 1.2 was found in the different lung lobes (Table 3). In group D, the distribution of surfactant was far more homogeneous over the total lung (Fig. 1*D*) but also over the different lung lobes (Table 3). A significantly higher percentage of lung pieces was found in the distribution interval 0.8 to 1.2 in the different lobes of the animals of group D compared with group B. In the animals of group E, there was a nonhomogeneous distribution (Fig. 1*E*), which was also represented by low percentages in the distribution interval 0.8 to 1.2 (Table 3).

^{99m}Tc scintigraphy. On the scintigrams, there was no difference between the radioactivity per area in the left and right hilar region and left and right peripheral lung region. We found that the distribution of both the ^{141}Ce microspheres and the ^{99m}Tc macroaggregated albumin in the lungs was the same. For each animal, we calculated a correlation coefficient of 0.96 between the distribution of ^{141}Ce microspheres and ^{99m}Tc macroaggregates

Table 2. *Partition of surfactant expressed by percentages of total ^{141}Ce counts over different lobes of lungs**

Group	Left upper lobe (%)	Left lower lobe (%)	Right upper lobe (%)	Right middle lobe (%)	Right lower lobe (%)
A	3.9 ± 2.3	28.5 ± 6.3	15.6 ± 5.6	3.9 ± 0.9	48.8 ± 4.8
B	8.9 ± 3.1	21.1 ± 2.8	16.0 ± 3.4	14.2 ± 2.9	39.8 ± 5.4
C	11.2 ± 1.9	29.2 ± 5.7	11.8 ± 2.2	13.4 ± 3.5	34.4 ± 1.5
D	11.9 ± 1.9	30.5 ± 1.3	12.1 ± 1.1	15.7 ± 1.8	29.7 ± 2.6
E	8.0 ± 2.7	16.7 ± 4.2	16.2 ± 2.3	17.4 ± 7.5	41.7 ± 5.7

* For definition of the groups, see Table 1. No significant differences were found between the percentages of the total ^{141}Ce counts for the different lung lobes in all rabbits independent of surfactant concentration or lung-lavage procedure.

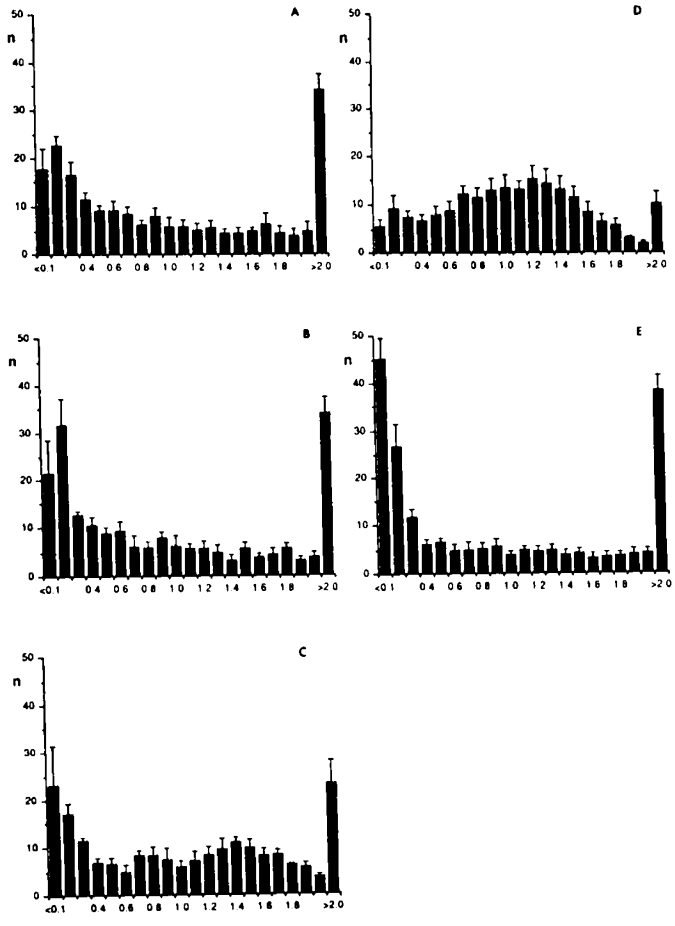

Fig. 1. Normalized distribution of exogenous surfactant for rabbits treated with different surfactant concentrations and volumes. For definition of groups, see Table 1. *A*, group A; *B*, group B; *C*, group C; *D*, group D and *E*, group E. All values were calculated as described in Materials and Methods and are expressed as mean number of pieces in each 10% distribution interval.

for the 200 pieces of lung tissue. The visual impression of the ^{99m}Tc scan suggested a homogeneous distribution, but the ^{141}Ce distribution histograms were similar to those found in Figure 1*B* (data not shown).

Arterial blood gas data. There was no significant difference in arterial P_{O_2}, P_{CO_2}, and pH measured in the groups before and after lung lavage. After the instillation of surfactant in a concentration of 45.0 g/L, the arterial oxygen tension increased only in group B from 10.3 ± 4.5 kPa to 18.2 ± 5.9 kPa. In the other groups, the arterial oxygen tension did not change after the instillation of surfactant.

DISCUSSION

In the present study, we demonstrated that the endotracheal instillation of 100 mg of surfactant/kg BW at a concentration of 45 g/L, as is clinically applied in newborn infants with RDS, results in a nonhomogeneous distribution to the lungs. This was found in normal rabbits and in rabbits with severe respiratory failure. We demonstrated that the distribution can be improved when this surfactant preparation is diluted with normal saline to a concentration of 6.25 g/L. Furthermore, we found a nonhomogeneous distribution when we administered 2.4 mL/kg BW (the volume of the surfactant preparation that is used clinically). Therefore, we conclude that the volume of fluid in which the surfactant is suspended is the major factor that determines the surfactant distribution to the lungs.

It could be argued that the flushing of the catheter with 1.5 mL of saline after surfactant administration would affect the distribution of surfactant differently in the groups receiving a low volume of surfactant. However, the amount of saline that was used for the removal of the surfactant residue in the catheter did not exceed the volume of the catheter and therefore it cannot be considered as an additional volume load to the lung.

We have shown that the technique used in this study is useful to differentiate between homogeneous and nonhomogeneous distribution of endotracheally administered surfactant. The amount of surfactant in each piece of lung tissue is related to the calculated mean. However, it is not possible to relate the amount of administered surfactant to the endogeneous surfactant pool size. Because we found similar distribution patterns in the rabbits with normal lungs (group A) and rabbits that underwent the lung-lavage procedure (group B), it is most unlikely that the distribution of administered surfactant is affected by the endogenous surfactant pool sizes. The impact of a calculated normalized value below 0.8 in pieces of lung tissue with respect to

Table 3. *Distribution of surfactant expressed as percentage of lung pieces with normalized value between 0.8 and 1.2 in different lobes of lungs and in total lung*

Group*	Left upper lobe (%)	Left lower lobe (%)	Right upper lobe (%)	Right middle lobe (%)	Right lower lobe (%)	Total lung (%)
A	11.3 ± 3.8	14.9 ± 1.5	18.8 ± 6.5	18.2 ± 1.7	32.1 ± 3.9†	12.4 ± 2.2
B	14.1 ± 1.8	10.3 ± 2.8	16.7 ± 3.8	15.2 ± 4.5	11.6 ± 4.4	12.6 ± 2.4
C	19.5 ± 2.9	16.0 ± 5.4	21.8 ± 7.8	18.2 ± 5.7	21.8 ± 4.0†	14.2 ± 2.8
D	29.9 ± 7.0	27.8 ± 5.4†	36.3 ± 5.1‡	26.5 ± 4.7	30.3 ± 4.3†	27.5 ± 3.6†
E	10.2 ± 1.6	6.7 ± 2.2	17.6 ± 3.8	12.4 ± 2.5	12.7 ± 4.1	9.2 ± 1.1

* For definition of the groups, see Table 1.
† $p < 0.05$ *vs* group B.
‡ $p < 0.01$ *vs* group B.

alveolar wall stability is not clear. Marks *et al.* (20) estimated that administered surfactant in a dose of 3.1 mg/kg BW should be adequate to promote alveolar stability. In animal studies, it has been shown that doses of 100 mg/kg BW in preterm baboons (21) and up to 170 mg/kg BW in immature lambs (22) had to be administered to improve lung function. Using our technique, it is impossible to relate the normalized values to a theoretical critical amount to achieve alveolar stability.

So far, few studies have been published on the distribution of exogenous surfactant in newborns with RDS. Ferrara *et al.* (23) studied the lungs of surfactant-treated patients who died of nonpulmonary causes. Fibrillar eosinophilic deposits in bronchi and alveoli, uniformly distributed over the different lung lobes, were considered to be the instilled surfactant. However, because no specific marker of the instilled surfactant was used, it has yet to be demonstrated that the fibrillar deposits do indeed stem from the instilled surfactant.

Charon *et al.* (19) used ^{99m}Tc scintigraphy to study surfactant distribution in newborn infants and stated that gross maldistribution could be ruled out by this technique, although these authors acknowledged that only a macroscopic impression of surfactant distribution could be obtained with this method. To assess the value of this technique, we compared ^{99m}Tc scintigraphy with our method of surfactant distribution. In a previous study, we demonstrated the reliability of ^{141}Ce microspheres to determine surfactant distribution. In that study, we showed a correlation of 0.96 between the distribution of cobalt-labeled microspheres and ^{14}C dipalmitoyl-phosphatidylcholine liposomes mixed with surfactant (17). Although the correlation of the microspheres and the dipalmitoyl phosphatidylcholine liposomes may not be the same in all lung pieces, from that research we concluded that mixing microspheres with the administered surfactant is an appropriate way to study the distribution of surfactant. In the present study, we found that the distribution of the ^{141}Ce microspheres and ^{99m}Tc macroaggregated albumin in the lungs was the same. However, although scintigraphy showed a homogeneous distribution, measurement of the radioactivity of the microspheres and the macroaggregated albumin in the small lung pieces both revealed a nonhomogeneous distribution. This implies that ^{99m}Tc scintigraphy has limited value in studying the distribution of exogenous surfactant in neonates.

In experimental studies, it has been demonstrated that the homogeneity of surfactant distribution is related to the fluid volume in which the surfactant is mixed. Gilliard *et al.* (14) found improvement of the distribution of surfactant by suspending the surfactant concentration in a larger fluid volume in normal rabbits but not in rabbits with injured lungs (14). Instillation of surfactant in a large fluid volume mimics the administration of surfactant to newborns before the first breath when the lungs are maximally filled with fetal lung fluid. When surfactant is administered before the first breath in premature lambs (15, 16) and rabbits (24), it will also be distributed relatively uniformly throughout the lungs in the fetal lung fluid. Of interest in this regard are the observations by Jobe *et al.* (25), in an experimental study, and by Enhörning *et al.* (26) and Smyth *et al.* (27), in human studies, that tracheal instillation of surfactant after a period of ventilation may lead to a clinical response of shorter duration than a treatment given at birth. A nonhomogeneous distribution of exogenously administered surfactant to the lungs may therefore contribute to the decreased clinical responses to surfactant therapy.

In case of surfactant deficiency, the bronchiolar and alveolar epithelial cells become damaged from repetitive overdistension during inspiration and collapse of the small airways at end-expiration (28, 29). It has been shown that regional variations in compliance in the lung exist, and that the bronchioli to lung parts with a low compliance are subjected to major shear forces (30). As a result, serum proteins leak into the alveoli, deactivate surfactant (31, 32), and trigger a vicious circle, so that gradually the number of affected terminal air spaces increases. The vicious circle of epithelial lesions, protein leakage, and surfactant inhibition can thus be amplified when surfactant is not distributed homogeneously to the lung. A homogeneous alveolar surfactant lining may prevent the development of multiple compliances in such a surfactant-deficient lung.

The administration of a large fluid volume with surfactant may have the advantage that only minimal pressures are necessary to expand the lungs to supplement the peripheral parts of the lung. From a theoretical point of view, fluid filling of the lungs eliminates the air-liquid interface, and, as a consequence, there will be no surface tension that has to be overcome by expansion of the lung. As early as 1929, von Neergaard (33) showed that it takes higher pressures to distend the lungs when filled with air than with water. He was the first to suggest that this difference was accounted for by the surface tension at the air-liquid interface inside the alveolar walls. Although we realize that the amount of fluid that was administered in the present study most likely did not fill all the alveoli, the homogeneous distribution indicates that a substantial number of the alveoli will be reached.

Although we demonstrate improved distribution of exogenous surfactant administrated in a large-volume fluid, we do acknowledge the fact that further studies are required to investigate the effects on lung function before introducing this treatment clinically. It seems, however, that introducing such a large fluid volume does not result in an immediate increase in the arterial P_{O_2}. We observed that the arterial P_{O_2} did not change 15 min after the administration of such a large fluid volume with surfactant, whereas the P_{O_2} was increased after the administration of a small-volume, high-concentration surfactant preparation. However, we have to realize that it may take some time before the fluid is absorbed and before the surfactant can exert its activity.

We conclude that although the administration of a surfactant preparation of 45 g of lipids/L results clinically in a rapid improvement of lung function, there is a nonhomogeneous distribution of this surfactant to the lung. This observation might at least in part explain the limited effect of surfactant therapy that is encountered in some patients. A significantly more homogeneous distribution can be obtained by increasing the fluid volume in which the surfactant is suspended. We speculate that the number of patients who do benefit from surfactant therapy will increase when the distribution can be improved. We demonstrate that dilution of the surfactant improves the distribution, but because we do not provide evidence on the safety and the improvement of lung function, we cannot recommend this treatment in patients at this stage.

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