Restoration of Lung Pressure-Volume Characteristics with Surfactant: Comparison of Nebulization Versus Instillation and Natural Versus Synthetic Surfactant

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Summary

The pressure-volume (PV) characteristics of 21 adult rat lungs were examined before and after lung washing after which natural or synthetic (dipalmitoyl lecithin (DPL)) surfactant was administered by either an ultrasonic nebulizer or instilled directly into the trachea. Lungs receiving surfactant by nebulization were ventilated at a rate of 40/min at 20 cm H₂O peak inspiratory pressure and with 7 cm H₂O positive end expiratory pressure (PEEP) for 2 hr. Lungs receiving surfactant by instillation were ventilated for 5 min in a fashion similar to the nebulization study; then a final PV curve was obtained.

Results from the deflation limb of the PV curves at 5 and 10 cm H₂O were analyzed and expressed as a percentage of the original total lung capacity obtained at 30 cm H₂O (percentage of TLC). The control values of percentage of TLC were 52.8 ± 6.4 at 5 cm H₂O and 73.9 ± 3.9 at 10 cm H₂O. Neither nebulization nor instillation of DPL produced a significant increase in percentage of TLC. Nebulization of natural surfactant produced only a slight increase in percentage of TLC from 4.8 ± 2.4 to 6.7 ± 2.1 (not significant) at 5 cm H₂O and from 21.1 ± 6.1 to 27.8 ± 5.4 ($P < 0.05$) at 10 cm H₂O. Instillation of natural surfactant produced a very striking increase of percentage of TLC from 13.2 ± 8.0 to 55.6 ± 4.3 ($P < 0.01$) at 5 cm H₂O and from 47.9 ± 6.1 to 78.6 ± 4.2 ($P < 0.01$) at 10 cm H₂O.

Speculation

From these studies, it appears that nebulization is not an effective method for depositing surfactant into the alveoli. On the other hand, direct instillation of the material into the tracheal bronchial tree seems to be a promising method worthy of further study as a method for treatment of the respiratory distress syndrome.

As the development of respiratory distress syndrome (RDS) is probably due to a prenatal deficiency of surfactant, one form of treatment might be the administration of surfactant through the airways. Several such attempts have been made experimentally and clinically. It has been shown that deposition of natural surfactant into the trachea of prematurely born rabbits before the first breath enhances the air expansion of their lungs (2). A favorable effect of nebulization of DPL was also demonstrated using excised dog lung (12), whereas clinical effects on infants with RDS by using an aerosol of DPL in Freon were inconsistent (1). This study was designed to determine whether the nebulization or instillation of surfactant (natural surfactant or DPL) may restore the PV characteristics of adult rat lungs in which surfactant was removed by saline washings.

MATERIALS AND METHODS

Adult male rats weighing 350-500 g were used in this study. Seven were used for pilot studies, 11 for the nebulization studies, and 10 for the instillation studies. After injecting 60 mg sodium barbital into the peritoneal cavity, each rat was killed by transecting the abdominal aorta and allowing exsanguination to occur. The heart and lungs were removed carefully en bloc.

PRESSURE-VOLUME MEASUREMENTS

The trachea was cannulated with polyethylene tubing and the lungs were degassed in a vacuum jar and placed in a saline water bath at 21°. The tracheal cannula was connected to a constant infusion syringe of 50 ml and to a water manometer by a T tube. The lungs were inflated with air gradually over 16-23 min to 30 cm H₂O in increments of 0.9 ml and then deflated in a similar fashion. The volume reached at 30 cm H₂O pressure was assumed as the total lung capacity. Pressure readings were made every minute to determine the volume on both inflation and deflation. Lungs that leaked were excluded.

A pressure-volume curve was derived from the pressure and volume measurements using a computer plotter Wang model 600. On the deflation limb of the pressure-volume curve, the volume at a pressure of 5 cm H₂O and 10 cm H₂O was expressed as a percentage of total lung capacity. The values of the volume were corrected by subtracting the volume displacement of the water manometer. Pressure-volume measurements were made three times on each lung: initially; after lung washing (removal of surfactant); and after treatment with surfactant.

REMOVAL OF SURFACTANT

After an initial pressure-volume curve recording, 10 ml of saline was infused into the lung and withdrawn gently several times through a cannula by hand. Fujiwara *et al.* (3) found that repeating this procedure 10 times produced a lung wash that was relatively free of surfactant on the 10th washing. Thus, to insure maximum removal of surfactant in our study, the procedure was repeated 20 times giving a total wash volume of 200 ml saline. At the end of the washings the effluent was clear and the pressure-volume curve showed poor stability. It was decided that lungs with volumes at 5 cm H₂O over 25% of total lung capacity after washings would be excluded from this study.

PREPARATION OF NATURAL SURFACTANT

The excised lungs of a calf 2 days old were washed with saline after exsanguination. Since the lung wash had a very large volume (2,500 ml), it was first centrifuged at 2° for 60 min at 11,000 rpm by using a Sorvall GSA rotor. The pellet was resuspended in cold saline, layered over 0.75 M sucrose (20 ml),

and then centrifuged at 20,000 rpm by using a Spinco SW25-1 rotor at 2° for 60 min. The material concentrating at the interface between saline and 0.75 M sucrose was aspirated by a disposable pipette and resuspended with saline in a volumetric flask. The total amount of phospholipid present was 596 mg. The phospholipid concentration of this solution was 2 mg/ml, and the minimal surface tension was 0 dynes/cm on a modified Wilhelmy balance.

SYNTHETIC SURFACTANT

Synthetic surfactant, supplied by Armour Pharmaceutical Company (Kankakee, Ill.), was diluted so that each ml contained 5 mg dipalmitoyl-dl-3-lecithin in buffered saline. Taken directly from the bottle, this suspension exhibited poor surface activity (*i.e.*, the minimal surface tension was 13 dynes/cm); however, when the suspension was extracted with chloroform and spread on the surface of saline, it showed high surface activity (*i.e.*, the minimal surface tension was 1 dyne/cm).

TREATMENT OF LUNGS

Four groups of animals were studied: nebulization with natural surfactant (five rats), nebulization with synthetic surfactant (six rats), instillation with natural surfactant (five rats), and instillation with synthetic surfactant (five rats).

In the nebulization groups, 40 ml surfactant suspension were used for each rat. For those receiving natural surfactant, the 40 ml contained 80 mg phospholipid and for those receiving synthetic surfactant, the 40 ml contained 200 mg DPL. In each experiment, the suspension was initially sonified by a Heat System Sonifier (model W-185E). The suspension was then placed into a 3 MHz LKB Medical Ultrasonic Nebulizer (model NB-108) and it was continuously nebulized into the lungs which were ventilated for 2 hr on a Harvard small animal respirator. The

Table 1. Total lung capacity before and after lung wash and treatment

Exp. no.	Initial, ml	After lung wash, ml	After treatment, ml	Treatment	Surfactant ¹
1	16.5	16.9	17.2	Instillation	Natural
3	14.0	13.0	8.5	Nebulization	Natural
5	8.5	6.7	5.1	Nebulization	Natural
6	17.5	16.1	12.8	Nebulization	Natural
7	15.7	13.3	10.8	Nebulization	Natural
8	19.0	16.4	15.7	Nebulization	DPL
9	15.4	10.0	7.0	Nebulization	Natural
10	17.1	12.4	10.0	Nebulization	DPL
11	15.2	11.6	8.5	Nebulization	DPL
12	19.6	13.7	4.6 ²	Nebulization	DPL
13	14.4	11.6	5.7 ³	Nebulization	DPL
14	5.4 ²	4.5 ²	4.2 ²	Nebulization	DPL
22	13.8	14.3	12.1	Instillation	Natural
23	15.6	11.9	15.6	Instillation	Natural
26	17.2	13.9	13.0	Instillation	Natural
27	14.6	12.3	10.9	Instillation	DPL
32	17.3	13.6	16.4	Instillation	Natural
33	17.8	15.9	18.3	Instillation	Natural
34	17.9	14.6	13.6	Instillation	DPL
35	15.5	13.7	13.3	Instillation	DPL
36	17.8	13.7	12.7	Instillation	DPL
Mean	16.0	13.3	12.3		
SD	± 2.4	± 2.3	± 3.6		

¹ Natural: natural surfactant; DPL: dipalmitoyl lecithin.

² Values are for left lung only; not used in calculation of mean ± SD.

³ Values are for left lower lobe only; not used in calculation of mean ± SD.

Table 2. Percentage of total lung capacity (TLC) of excised rat lungs at 5 and 10 cm H₂O before and after nebulization of natural surfactant

Exp. no.	% TLC at 5 cm H ₂ O				% TLC at 10 cm H ₂ O			
	Initial	After wash	After treatment	Change	Initial	After wash	After treatment	Change
3	62.5	6.3	10.0	+3.7	80.9	23.1	33.1	+10.0
5	48.1	5.6	7.5	+1.9	70.6	18.1	20.6	+2.5
6	55.6	2.8	6.2	+3.4	75.0	23.1	29.4	+6.3
7	52.5	7.5	4.4	-3.1	71.6	28.8	32.2	+3.4
9	47.5	1.9	5.6	+3.7	71.6	12.5	23.8	+11.3
Mean	52.2	4.8	6.7	+1.9	73.9	21.1	27.8	+6.7 ¹
SD	± 6.1	± 2.4	± 2.1	± 2.9	± 4.2	± 6.1	± 5.4	± 3.9

¹ $P < 0.05$.

Table 3. Percentage of total lung capacity (TLC) of excised rat lungs at 5 and 10 cm H₂O before and after nebulization of dipalmitoyl lecithin

Exp. no.	% TLC at 5 cm H ₂ O				% TLC at 10 cm H ₂ O			
	Initial	After wash	After treatment	Change	Initial	After wash	After treatment	Change
8	48.1	3.8	8.1	+4.3	67.2	24.1	30.9	+6.8
10	62.5	10.6	7.5	-3.1	80.0	36.9	37.5	+0.6
11	55.6	12.5	5.0	-7.5	76.2	34.1	23.8	-10.3
12	69.4	11.9	6.9	-5.0	80.6	26.9	19.1	-7.8
13	52.5	12.0	4.0	-8.0	72.8	27.5	16.2	-11.3
14	48.8	5.0	6.9	-1.9	69.0	13.1	22.2	+9.8
Mean	56.2	9.3	6.4	-2.9	74.3	27.1	25.0	-2.2
SD	± 8.3	± 3.9	± 1.6	± 5.0	± 5.6	± 8.4	± 7.9	± 8.9

Table 4. Percentage of total lung capacity (TLC) of excised rat lungs at 5 and 10 cm H₂O before and after instillation of natural surfactant

Exp. no.	% TLC at 5 cm H ₂ O				% TLC at 10 cm H ₂ O			
	Initial	After wash	After treatment	Change	Initial	After wash	After treatment	Change
1	60.4	5.4	53.9	+48.5	75.0	50.0	71.9	+21.9
22	43.1	18.8	59.4	+40.6	68.8	51.3	81.9	+30.6
23	50.0	24.4	56.9	+32.5	75.0	46.3	80.0	+33.7
32	51.3	8.1	48.8	+40.7	78.1	38.1	77.5	+39.4
33	55.6	9.4	58.8	+49.4	77.5	53.8	81.9	+28.1
Mean	52.1	13.2	55.6	+42.3 ¹	74.9	47.9	78.6	+30.7 ¹
SD	±6.5	±8.0	±4.3	±6.9	±3.7	±6.1	±4.2	±6.5

¹ $P < 0.01$.Table 5. Percentage of total lung capacity (TLC) of excised rat lungs at 5 and 10 cm H₂O before and after instillation of dipalmitoyl lecithin

Exp. no	% TLC at 5 cm H ₂ O				% TLC at 10 cm H ₂ O			
	Initial	After wash	After treatment	Change	Initial	After wash	After treatment	Change
26	45.6	18.8	18.8	0	71.3	50.0	50.0	0
27	49.4	13.4	15.0	+1.6	75.0	44.5	45.9	+1.4
34	48.8	14.1	16.9	+2.8	70.6	51.9	53.8	+1.9
35	49.4	21.3	21.3	0	71.9	53.8	53.8	0
36	51.3	13.4	11.9	-1.5	74.4	50.0	49.4	-0.6
Mean	48.9	16.2	16.8	+0.7	72.6	50.0	50.6	+0.8
SD	±2.1	±3.6	±3.6	±1.9	±1.9	±3.5	±3.3	±1.0

ventilation rate was 40/min, the maximum inspiratory pressure was 20 cm H₂O, and the PEEP was 7 cm H₂O. The nebulized fog produced with both DPL and natural surfactant when spread on a saline-filled trough of a modified Wilhelmy balance gave minimum surface tension below 10 dynes/cm.

In the instillation groups, 2 ml surfactant suspension were instilled into the trachea of each rat after which the lungs were ventilated for 5 min on the respirator in a fashion similar to the nebulization studies. For those receiving natural surfactant, the amount instilled contained 4 mg phospholipid and for those receiving synthetic surfactant, the amount instilled contained 10 mg DPL.

RESULTS

The assumed TLC's of adult rat lungs (the volume at a pressure of 30 cm H₂O) are listed in Table 1. The mean initial TLC was 16.0 ml and after the lung washing, the TLC decreased to 13.2 ml. Eight of the lungs were weighed immediately after removal from the rat. The mean lung weight was 1.89 ± 0.35 g. When the initial total lung capacities (*i.e.*, before washing or treatment) were expressed as TLC/lung weight, a mean of 9.1 ± 1.6 ml/g lung tissue was obtained.

The results of the lung pressure-volume characteristics during deflation at 5 cm H₂O and 10 cm H₂O before and after the nebulization and instillation of surfactant are shown in Tables 2-4. As shown in Table 2, the mean percentage of TLC before the nebulization of natural surfactant was 4.8 ± 2.4 at 5 cm H₂O and 21.1 ± 6.1 at 10 cm H₂O. The mean percentage of TLC after nebulization of natural surfactant was 6.7 ± 2.1 at 5 cm H₂O and 27.8 ± 5.4 at 10 cm H₂O. The mean difference between before and after nebulization of natural surfactant was $+1.9 \pm 2.9$ at 5 cm H₂O and $+6.7 \pm 3.9$ at 10 cm H₂O. Thus, although nebulization of natural surfactant increased the percentage of TLC at 5 cm H₂O and 10 cm H₂O ($P < 0.05$), the increase was small.

As shown in Table 3, the mean percentage of TLC before nebulization of DPL was 9.3 ± 3.9 at 5 cm H₂O and 27.1 ± 8.4

at 10 cm H₂O. The mean percentage of TLC after nebulization of DPL was 6.4 ± 1.6 at 5 cm H₂O and 25.0 ± 7.9 at 10 cm H₂O. The mean difference between before and after nebulization of DPL was -2.9 ± 5.0 at 5 cm H₂O and -2.2 ± 8.9 at 10 cm H₂O, which was not significant.

As shown in Table 4, the mean percentage of TLC before instillation of natural surfactant was 13.2 ± 8.0 at 5 cm H₂O and 47.9 ± 6.1 at 10 cm H₂O. The mean percentage of TLC after instillation of natural surfactant was 55.6 ± 4.3 at 5 cm H₂O and 78.6 ± 4.2 at 10 cm H₂O. The mean difference between before and after instillation of natural surfactant was 42.3 ± 6.9 at 5 cm H₂O ($P < 0.01$) and 30.7 ± 6.5 at 10 cm H₂O ($P < 0.01$). Instillation of natural surfactant produced a change in percentage of TLC that was significantly greater than that produced by nebulization of natural surfactant at both 5 and 10 cm H₂O ($P < 0.01$). Therefore, the instillation of natural surfactant significantly improved the pressure-volume characteristics of surfactant-depleted lung.

Fig. 1. A comparison of the percentage of total lung capacity at 5 cm H₂O for control lungs, lungs washed with 200 ml saline, and washed lungs that were treated with natural surfactant and synthetic, dipalmitoyl lecithin (DPL).

As shown in Table 5, the mean percentage of TLC before instillation of DPL was 16.2 ± 3.6 at 5 cm H₂O and 50.0 ± 3.5 at 10 cm H₂O. The mean percentage of TLC after instillation of DPL was 16.8 ± 3.6 at 5 cm H₂O and 50.6 ± 3.3 at 10 cm H₂O. The mean difference between before and after instillation of DPL was 0.7 ± 1.9 at 5 cm H₂O and 0.8 ± 1.0 at 10 cm H₂O. The pressure-volume characteristics did not change significantly after instillation of DPL.

A comparison of the results obtained in the four different treatment groups is shown in Figure 1. It indicates that the instillation of natural surfactant is the best way to restore the pressure-volume characteristics when compared with the other three methods.

DISCUSSION

This study has demonstrated that the instillation of natural surfactant into the lungs can restore the pressure-volume characteristics of adult rat lung previously depleted of surfactant by saline wash. Others have shown that saline lung lavage removes surfactant from the alveoli (3, 8). The PV characteristics of the surfactant-depleted rat lungs used in this study were similar to those in the lungs of infants dying of hyaline membrane disease (HMD) reported by Gribetz *et al.* (7). They found that the volumes at 5 cm H₂O and 10 cm H₂O on the deflation limb of the pressure-volume curve of HMD lungs were 0.4 ml/g and 0.5 ml/g respectively, whereas the volumes of normal newborn were 1.9 ml/g and 2.2 ml/g. In rat 33 of our study (as shown in Figure 2), the volumes at 5 cm H₂O and 10 cm H₂O were 0.5 ml/g and 2.5 ml/g, respectively, after lung wash and 4.3 ml/g and 6.2 ml/g before wash when expressed in the same scale. It seems, therefore, that adult rat lungs depleted of natural surfactant by saline wash may be a good experimental model of the surfactant-deficient lungs of prematurely delivered infants.

Chu *et al.* (1) attempted to improve the ventilation of infants with RDS with an aerosol of DPL in Freon, but the results were inconsistent. Shannon *et al.* (12), however, reported that the lecithin fog generated by an ultrasonic nebulizer (frequency 3 MHz) restored the pressure-volume characteristic of adult dog lung. For this reason, we used an LKB high frequency 3-MHz ultrasonic nebulizer to aerosolize the surfactant suspension.

In the study of Shannon *et al.* (12), the excised dog lungs were ventilated for 3 hr to decrease lung surface activity. They expressed the change in volume over the fall in pressure from 8 to 3 cm H₂O, and showed a 50% change in slope after ventilation with DPL fog. In contrast, our experiments did not show improvement of pressure-volume curves after ventilation with nebulized DPL. The discrepancy between our study and their study may be due to the difference in the technique of depletion of lung surfactant. If the depletion of surfactant was incomplete in Shannon's study, then the recovery of surface activity

might be easily accomplished by DPL nebulization. We believe our saline wash technique more effectively removed surfactant and thus produced less compliant lungs than did Shannon's technique. Thus, the greater instability of the washed lungs in our study may have prevented deposition of DPL by nebulization.

It has been suggested that the depletion of the lung surface-active lining layer is related to catabolism (5) or to disruption of the surface lining layer (5, 9). Repeated overinflation of excised lungs reduces compliance by an amount which is directly related to the tidal volume and to the duration and frequency of ventilation (5, 6, 9). We therefore used a relatively low inflation pressure of 20 cm H₂O and an end expiratory pressure of 7 cm H₂O to prevent the deterioration of the lining layer. We observed significant deterioration (7%) of the deflation slope of the pressure-volume curve after 2 hr of ventilation with air, thus confirming the studies of Forest (6) and Pattle (10). The improvement of the pressure-volume characteristics of the lung by natural surfactant nebulization may therefore have been underestimated because of the deleterious effects of ventilation.

The favorable effects of tracheal instillation of natural surfactant were first reported by Enhörning *et al.* (2). They found that the lung expansion of the premature rabbit fetus can be enhanced by tracheal deposition of homologous surfactant before the first breath. Premature fetuses given surfactant showed pressure-volume characteristics similar to those of full term fetuses. These studies have now been expanded to include various techniques for surfactant deposition in live born premature rabbits (11). Our study on surfactant-depleted adult rat lungs seems to confirm their results.

Using the value of 7 cm²/g body weight (13) for the lung surface area, we found that the surface area of the lung of a 500-g rat is 3,500 cm². Since Fujiwara *et al.* (4) determined that the amount of DPL per unit surface area required to produce stable lungs was 5.7×10^{-4} mg/cm², we calculated that about 2 mg DPL were required to replace the surfactant of the washed rat lungs. We showed in our study that instillation of a natural surfactant solution containing 4 mg total phospholipids (2 mg DPL) was enough to restore the pressure-volume characteristics of washed lungs. Instillation of 10 mg synthetic DPL, however, did not succeed in restoring pressure-volume characteristics. The discrepancy in efficacy of DPL compared to natural surfactant could be a result of the properties of the solvent used with DPL suspension or a lack of some other compound which is present in the natural surfactant suspension.

Our method of delivering natural surfactant to the lungs offers several practical and theoretical advantages that might enhance correction of alveolar surfactant deficiency. More studies will be necessary to determine the physical and chemical differences between natural surfactant and synthetic DPL.

CONCLUSION

Excised rat lungs from which surfactant was removed by washing with saline were treated by four different methods in an attempt to restore the PV characteristics to normal. The four methods were: nebulization of DPL, instillation of DPL, nebulization of natural surfactant, and instillation of natural surfactant. Results were analyzed from the deflation limb of the PV curve and expressed as percentage of TLC.

Using the techniques employed in this study, instillation of natural surfactant was the only method which restored the PV characteristics of surfactant-depleted lungs to normal.

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Fig. 2. Pressure-volumes (PV) curves on rat 33.

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Aldosterone newborn
diet weight
kidney

Plasma Aldosterone Concentrations during the Neonatal Period

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Summary

The direct radioimmunoassay for plasma aldosterone (PA) set up in this laboratory has been reported elsewhere. Subjects were 115 normal full term newborns and infants. During the first week of life, there was a wide dispersion in the PA levels ($<1-445$, $n = 89$), but on the whole these levels were high (>6 ng/100 ml) and 35% were higher than those observed in children after stimulation (>48 ng/100 ml). Furthermore, there was a negative correlation between PA values and the day of sampling ($P < 0.01$). In older infants, PA values were lower and the ranges narrower. A highly significant negative correlation was found between Na:K ratios and PA levels. The mean geometrical value for PA levels was 53 ng/100 ml vs. 1.012 for Na:K mean ratio. When related to weight variations, PA values were randomly distributed. No correlation was found between PA and sodium intakes. The daily urinary excretion of tetrahydroaldosterone and 3-oxoconjugate of aldosterone were lower *per se*, but when related to surface area, were found to be roughly 3-fold those of control adult subjects. We conclude that in the newborn, both the production and excretion of aldosterone are different than values in the adult and that responses to the regulation factors mentioned above are decreased.

Speculation

The direct radioimmunoassay of PA facilitates the evaluation of this hormone during the neonatal period and infancy. The results obtained from plasma and urinary determinations of aldosterone provide evidence for a different pattern of both production and metabolism of aldosterone during these periods. An appreciation of these PA values and of their regulation is necessary in order to evaluate the significance of PA levels observed in pathologic cases.

It has previously been reported that PA levels are dramatically elevated during the neonatal period (3, 11) and infancy (5, 8). However, apart from this common point, the results obtained have been widely dispersed and all authors have found values overlapping those observed in the adult. We undertook the present study in order to obtain further information on PA levels, especially during the neonatal period, and to try and relate the results we obtained with a physiologic index of aldosterone activity or regulation.

METHODS

METHOD FOR MEASUREMENT OF PA

The direct radioimmunoassay set up in this laboratory has been reported elsewhere (11). One milliliter of plasma was extracted with 10 ml methylene chloride and the extract washed first with 1 ml of 0.1 M NaOH and then with 1 ml distilled water. Different aliquots of the extract, determined according to the expected aldosterone concentrations, were assayed without further purification with the aid of a highly specific antibody. The sensitivity of the assay was 1 ng/100 ml plasma. Some plasma samples from newborns were submitted to Sephadex LH20 chromatography as described by Murphy (9), using a methylene chloride-methanol (98:2) solvent.

URINARY METABOLITES OF ALDOSTERONE

These were assayed by radioimmunoassay after column chromatography.

SUBJECTS

The subjects were 115 normal full term newborns and infants. Blood samples were taken from the antecubital vein of the