Evaluation of Lung Function Changes Before and After Surfactant Application During Artificial Ventilation in Newborn Rats With Congenital Diaphragmatic Hernia

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● Patients with congenital diaphragmatic hernia (CDH) have unilateral or bilateral hypoplasia of the lungs including delayed maturation of the terminal air sacs. Because these lungs are highly susceptible to barotrauma and oxygen toxicity, even in full-term newborns, continued research into optimal ventilatory regimen is essential to improve survival rate and to prevent ongoing lung damage. Against this background, the effect of exogenous surfactant application is evaluated. In newborn rats, CDH was induced after a single dose of 2,4 dichloro-4'-nitrophenyl (Nitrofen) (400 mg/kg) on day 10 of gestation. The newborn rats were intubated immediately after hysterotomy, transferred to a heated multichambered body plethysmograph, and artificially ventilated. Inspiratory peak pressures were initially set at 17 cm H_2O, with positive end-expiratory pressure at 0 cm H_2O and FIO_2 at 1.0. The pressure was raised in steps of 5 cm H_2O, from 5 to 30 cm H_2O, to obtain pressure-volume diagrams at 0, 1, and 6 hours of artifical ventilation. These measurements were obtained in controls and in CDH rats with and without endotracheal installation of bovine surfactant (n = 4 to 10 in each group). Significant differences in lung volume between CDH and control rats were observed at all time-points. Surfactant application had a positive effect on lung volume, especially in control rats at t = 1 hour. No significant differences were observed between the CDH groups at t = 1 or t = 6 hours. In this animal model, the effect of artificial ventilation as well as the beneficial short-term effect of exogenous surfactant application have been evaluated. A continued positive effect on lung volume in CDH lungs could not be determined. Routine administration of exogenous surfactant in human CDH patients is not supported by these experimental results.

INDEX WORDS: Diaphragmatic hernia, congenital, newborn, artificial ventilation, pulmonary hypoplasia, surfactant.

IN PATIENTS who have congenital diaphragmatic hernia (CDH), abnormal morphological development of both lungs and the intrapulmonary blood vessels is present.[1-5] However, reports on the biochemical maturation of the lung in CDH are scarce.

Hisanaga et al[6] noted low lecithin:sphingomyelin ratios (0.56 and 0.57, respectively) in two infants with CDH. They argued that lung hypoplasia actually means a reduced number of type II pneumocytes and reduced production of surfactant. The lecithin: sphingomyelin ratio reflects to some extent the maturation of the fetal lung. However, the question remains: are the ratios low because of the total reduction in lung tissue or because the type II cell in CDH functions at a lower level?[7]

Hashimoto et al[8] investigated the morphological characteristics of the type II cell in a fetal lamb model of CDH. Surprisingly, they found that type II cells were 5 to 10 times more abundant in the lungs of animals with a diaphragmatic defect. No ultrastructural changes of immaturity were observed in type II cells.

Glick et al[9] studied lung surfactant production in the fetal lamb after experimental induction of CDH and showed (1) a marked decrease in pulmonary compliance, (2) a reduction of the total amount of phospholipid in bronchoalveolar lavage fluid, and (3) a reduction in the synthesis rate of phosphatidylcholine by type II cells. The authors concluded that CDH in the fetal lamb leads to profound lung hypoplasia and apparent immaturity of the surfactant system.

In neonatal rats, CDH can be induced by feeding of 2,4 dichloro-4'-nitrophenyl ether (Nitrofen, Rohm & Haas Co, Philadelphia, PA) to the mothers during gestation, as documented previously.[10-12] In this model, the lungs of CDH animals showed hypoplasia and lower content of disaturated phosphatidylcholine per microgram DNA and total disaturated phosphatidylcholine, as shown by Suen.[13]

The conflicting results obtained in different animal models has guided us to investigate the effect of exogenous surfactant application in relation to changes in lung volume after artificial ventilation in newborn rats with CDH.

MATERIALS AND METHODS

Female Sprague Dawley rats (Harlan, Zeist, The Netherlands) weighing 240 to 280 g were mated during 1 hour (day 0 of gestation). On day 10 of gestation, 100 mg of 2,4 dichloro-4'-nitrophenyl ether (Nitrofen), dissolved in 1 mL of olive oil, was

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administered by gastric tube to the Nitrofen group; controls received 1 mL of olive oil. Food and water were supplied ad libitum during the entire period. Hysterotomy was performed on gestational day 22.

The animals born spontaneously were excluded from the experiment because the period of spontaneous breathing could not be determined in detail and would influence lung function parameters. There were three groups of newborns: controls, animals with a diaphragmatic hernia, and animals without a diaphragmatic defect after Nitrofen administration (10%-20% of the animals receiving Nitrofen).

Before the hysterotomy, the dam was anaesthetized with N_2O and enflurane-inhalation, and the fetuses were delivered. Immediately after hysterotomy, the newborns were weighed and received pancuronium bromide (0.08 mg/kg each second hour) and pentobarbital (40 mg/kg each third hour) intraperitoneally, followed by intubation with a metal canula. The cannulas used throughout the experiment were made from syringe needles (internal diameter, 0.5 mm; external diameter, 0.7 mm).

The intubated animals were immediately transferred to a multichambered, pressure-constant body plethysmograph heated to 38°C. This procedure lasted 1 to 2 minutes for each animal. A flexible tube provided an adequate connection between the trachea and the body-box. The maximum number of ventilated animals per litter was nine.

Artificial Ventilation

The body plethysmograph was connected to a modified ventilator (Servo 900B; Siemens-Elema, Solna, Sweden), as routinely used in the I.C.U., and as described by Lachmann et al.[14] This equipment provides pressure-generated ventilation with decelerating flow, using excess flow through the ventilator system.

The ventilator settings throughout the experiment were as follows: FIO_2, 1.0; frequency, 40/min; I:E ratio, 1:2; inspiratory peak pressure, 17 cm H_2O; positive end-expiratory pressure (PEEP), 0 cm. These settings were changed only to obtain pressure-volume relations, ie, the pressures were raised from 7 cm H_2O, in steps of approximately 5 cm H_2O, to a maximum of 30 cm H_2O. The other settings remained the same throughout the experiments. The pressure-volume relations were determined for each fetus with a specially designed Fleisch-tube[14] connected to the body plethysmograph, a differential pressure transducer (EMT 34; (Siemens-Elema) and amplifier (EMT 31), an integrator unit (EMT 41), and a recorder Mingograf 81; Siemens-Elema).

The animals were ventilated for a maximum of 6 hours. Pressure-volume relations were determined at 0, 1, and 6 hours. Zero hours applied to the animals that were intubated immediately after hysterotomy because they showed no signs of spontaneous breathing.

The animals without visible heart action, pneumothorax, or other complications related to insufficient ventilation or technical problems were excluded from the study. The same holds true for animals without CDH after Nitrofen administration and those with huge diaphragmatic defects that died less than 6 hours after birth. After the experiments, the animals were killed by an overdose of pentobarbital.

Surfactant Application

After intubation and the start of artificial ventilation (with peak inspiratory pressures of 17 cm H_2O, PEEP of 0 cm H_2O, and FIO_2 of 1.0), both control and Nitrofen animals received either a bolus of bovine surfactant (0.05 mL of a 25-mg/mL solution) or nothing. Pressure-volume diagrams were obtained as described before, at t = 1 and t = 6 hours.

Calculations

By means of the measured pressure-volume points, a pressure-volume curve was plotted for each animal at each time-point. The volume at the standardized pressures of 7, 10, 15, 20, 25, and 30 cm H_2O was determined from these curves and recorded. For each group, the means of volume were calculated at standardized pressures. These means were compared, and statistical significance was determined by means of the Mann-Whitney or Student's *t* test.

RESULTS

For each group, the values of lung volume at the peak pressures of 15 and 25 cm H_2O (with standard deviation) as well as the number of animals at each time-point are shown in Table 1. Representative pressure-volume diagrams are shown in Figs 1 and 2.

Table 1. Pressure/Volume Relations in Control and CDH Rats at Different Times, With or Without Exogenous Surfactant

		Control					Hernia			
Time (hours)		No Surfactant		Surfactant			No Surfactant		Surfactant	
		15 cm H_2O	25 cm H_2O	15 cm H_2O	25 cm H_2O		15 cm H_2O	25 cm H_2O	15 cm H_2O	25 cm H_2O
0	Mean	24.8	81.4	—	—	Mean	5.9	22.4	—	—
	SD	17.6	32.1			SD	5.5	23.6		
	n	15				n	9			
	Significance					Significance	#	#		
1	Mean	109.8	160.4	108.6	216.9	Mean	34.1	95.3	26.9	83.5
	SD	34.9	41.1	59.2	61.9	SD	13.9	38.2	17.1	20.6
	n	9		12		n	6		6	
	Significance	@	@	@	@*	Significance	@#	@#	@#	@#
6	Mean	141.3	165.1	146.5	190.4	Mean	41.6	87.8	44.9	85.6
	SD	50.8	63.3	45.9	61.5	SD	27.3	25.3	30.4	38.6
	n	8		10		n	6		4	
	Significance	@	@	@	@	Significance	@#	@#	@#	@#

NOTE. For all values, $P < 5\%$.

Abbreviations: @, significant from T = 0 in the same group; *, significant from the same group without surfactant; #, significant from control at the same time.

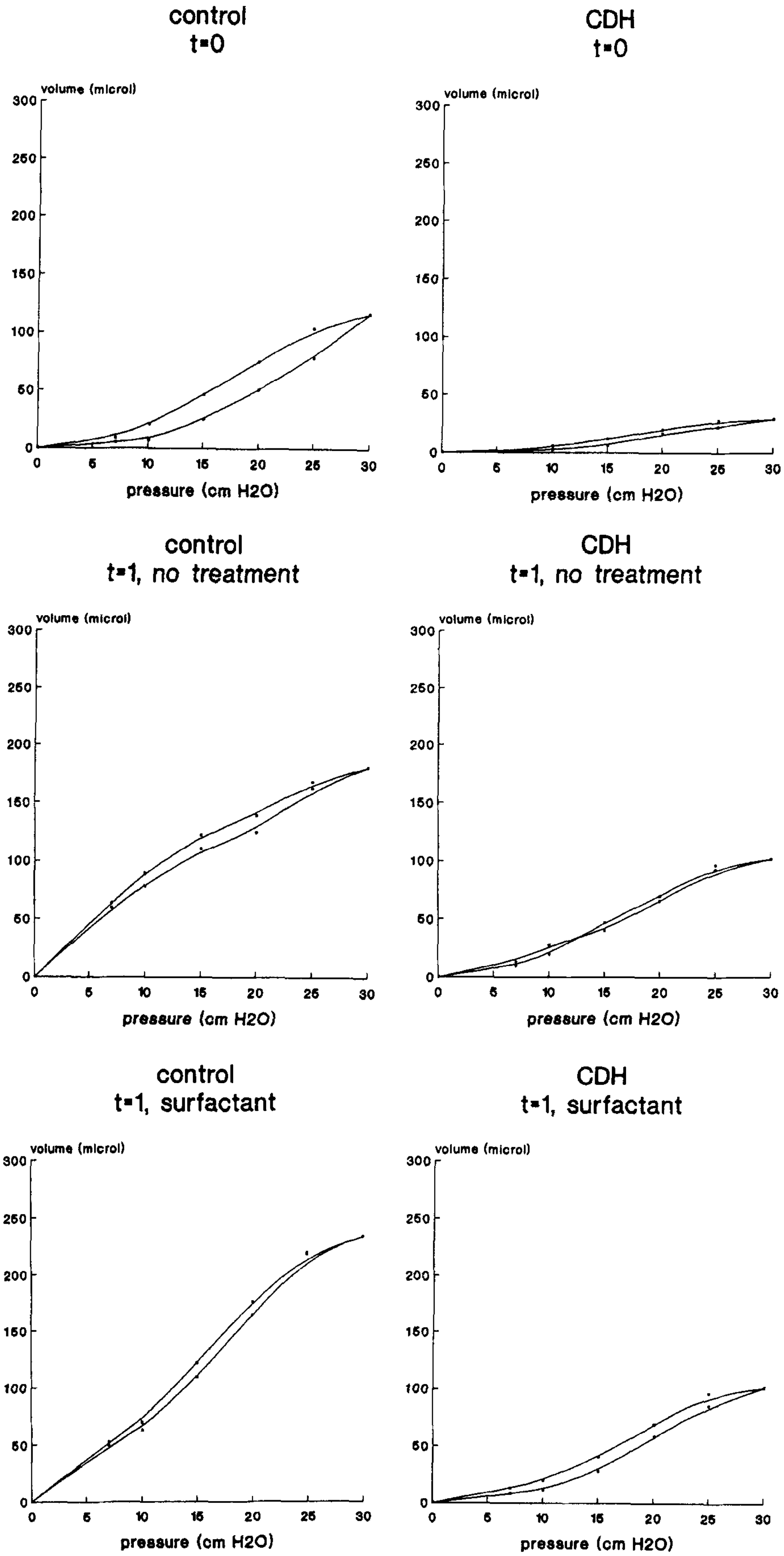

Fig 1. Representative pressure-volume curves at t = 0 and t = 1 in control rats, CDH rats without treatment, and CDH rats with surfactant.

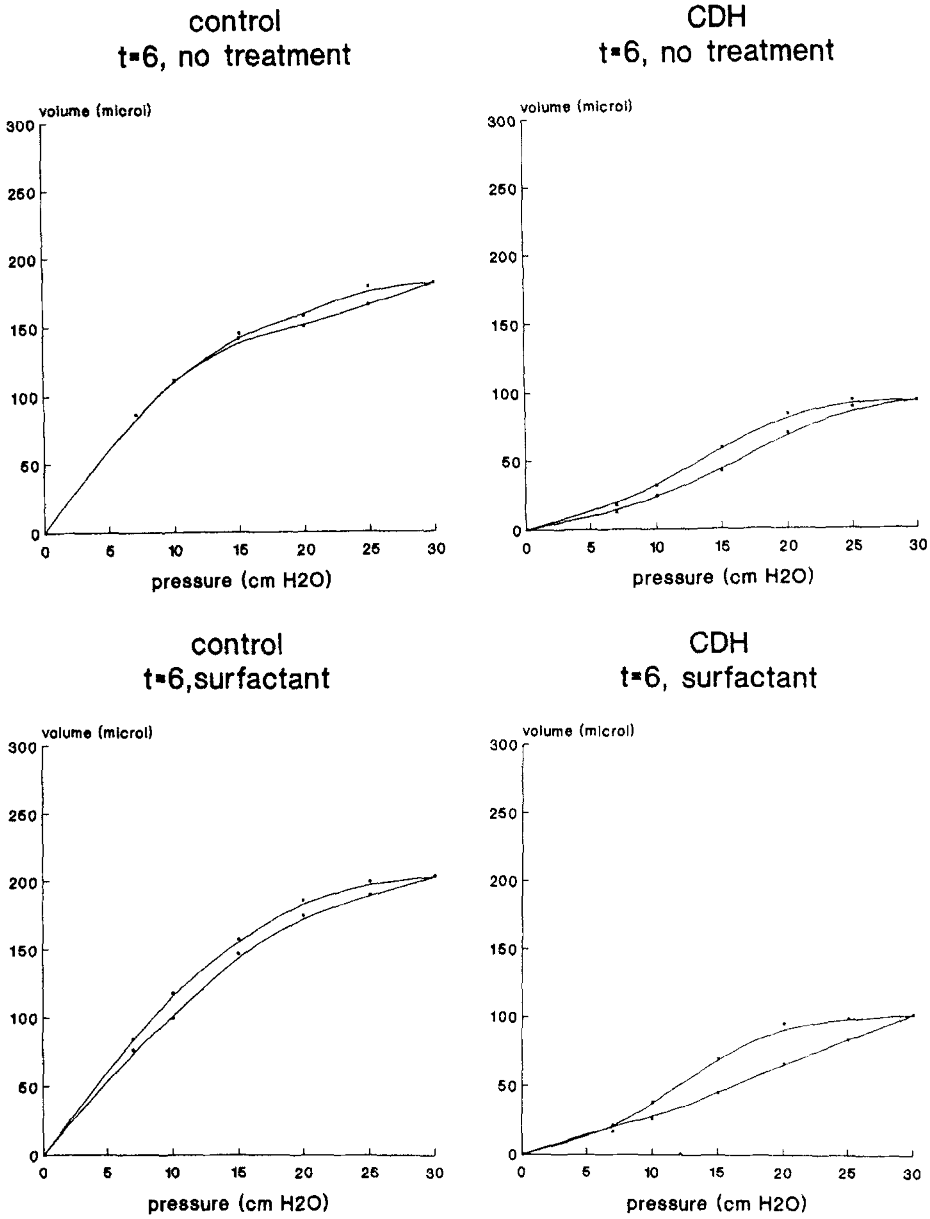

Fig 2. Representative pressure-volume curves at t = 6 in control rats, CDH rats without treatment, and CDH rats with surfactant.

There are significant differences for each time-point between control and CDH rats, with CDH rats having lower lung volumes.

The effect of superfactant application is greater in controls than in CDH rats. A positive effect of surfactant application in control rats was found at t = 1 hour. There were no significant differences at t = 1 or t = 6 hours between the CDH groups.

DISCUSSION

Animal Data

Our study shows not only that artificial ventilation of neonatal rats is possible but also that the effect of exogenous surfactant application can be studied in detail. Following the classic approach of inducing CDH in sheep (by Harrison et al[15]), Hashimoto et al[8] investigated the morphological characteristics of the type II cell in a fetal lamb model of CDH. Surprisingly, they found that type II cells were 5 to 10 times more abundant in the lungs of animals having a diaphragmatic defect. No ultrastructural changes of immaturity were observed in type II cells.

In contrast to the above-mentioned study, various other research groups have documented or suggested that in the hypoplastic lungs of CDH rats, immaturity of the lungs exists.[12,13,16] Suen et al,[13] using whole-lung homogenates, noted significantly lower desaturated phosphatidylcholine (DSPC) per microgram DNA and total DSPC in CDH rats, and Brandsma et al,[16] using bronchoalveolar lavages of control and CDH rats, came to the same conclusion. Recently, Suen et al[17] documented the positive effect of antenatal glucocorticoid treatment on the DSPC content of whole-lung homogenates in rats with Nitrofen-induced CDH.

The reason we were not able to demonstrate a continuing positive effect on lung volume in CDH rats might be related to the method of delivery, dosage, timing, or volume of surfactant application; the effect of introducing PEEP should be determined as well.

Human Data

Increasing evidence shows that the hypoplastic lung in CDH is developmentally retarded.[1-3] A lower lecithin-sphingomyelin (L/S) ratio in the amniotic fluid[6] as well as morphological findings showing hyaline membranes in full-term infants with CDH emphasize the significance of a further characterization of the developing terminal lung unit in these patients. For a patient with CDH, accurate determination of the presence of a surfactant deficiency, either primary or secondary, will be of great significance in the selection of appropriate treatment modalities.[18] Moreover, the high incidence of bronchopulmonary dysplasia in surviving patients[19] might be prevented by early administration of surfactant, either prophylactically or therapeutically, as has been shown for premature infants with respiratory distress syndrome.[20]

Treatment of CDH can be individualized by using standard procedures such as bronchoalveolar lavages, as documented by Stenmark et al,[21] newborns with persistent pulmonary hypertension, to detect surfactant deficiency. Prospective randomized trials in CDH patients, using exogenous surfactant as either prophylaxis or rescue therapy, should be undertaken in the near future to test the value of this approach.

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