Pediatric Pulmonology 27:104–112 (1999)

Changes in Pulmonary Function in Preterm Infants Recovering From RDS Following Early Treatment With **Ambroxol:** Results of a Randomized Trial

Gerd Schmalisch, DScNat[1]* Roland Reinhard Wauer, MD,[1] and Bernd Böhme, MD[2]

Summary. Several studies have demonstrated that ambroxol stimulates surfactant synthesis and has antioxidative and antiinflammatory effects. We investigated the effect of ambroxol on lung function in newborns with respiratory distress syndrome (RDS) weighing <1,500g. In all, 102 newborns were enrolled (52 received ambroxol and 50 placebo). After extubation, lung function tests were performed weekly using a face mask for ventilatory measurements and a catheter tip pressure transducer (diameter 1.7 mm) for esophageal pressure measurements (P_{es}). The flow-through technique was used to eliminate apparatus dead space and to allow long-term measurements during quiet sleep. Percentile curves of pulmonary function parameters from healthy newborns were used for comparison.

During the first 28 days, 42 newborns were extubated in the ambroxol group and 36 in the placebo group. The ventilatory parameters of both treatment groups were in the normal range and there were no significant differences between the two groups at any time. After extubation, the ratio of tidal volume to maximal esophageal pressure changes (V_T/ $P_{es,max}$) was below the 10th percentile in the ambroxol and placebo-treated groups. In the ambroxol group the 10th percentile was reached on day 10, whereas in the placebo group the 10th percentile was reached significantly later ($P < 0.05$) on day 23. Modeling of power expenditures was used to identify the optimal breathing pattern so that small differences in ventilatory parameters between the two groups could be analyzed.

We conclude that early ambroxol treatment has only a modest effect on lung function in newborns with established RDS. The sensitivity of tidal breathing parameters is not sufficient to detect these small changes in lung mechanics, but small improvements could be demonstrated in lung mechanics 10 days after extubation in the ambroxol-treated group. **Pediatr Pulmonol. 1999; 27:104–112.**

Key words: ambroxol; respiratory distress syndrome; surfactant induction; lung function testing; regulation of breathing; newborns.

INTRODUCTION

Since the end of the 1950s, it has been known that the respiratory distress syndrome (RDS) in premature infants is caused by a deficiency or a dysfunction of pulmonary surfactant.[1] There are potentially three pharmacological possibilities that may help to increase the depleted alveolar pool size of surfactant postnatally in infants with RDS: 1) endotracheal instillation of exogenous surfactant, 2) enhancement of endogenous surfactant biosynthesis and secretion by type II cells, and/or 3)inhibition of surfactant clearance.

Numerous studies have demonstrated the efficacy of surfactant replacement therapy in RDS,[2–5] but only a few studies have tested the clinical efficacy of drugs that stimulate the synthesis or release of endogenous surfactant.[6–8]

Previous studies on fetal animals[9] have shown that ambroxol (ambroxolhydrochlorid:trans-4-[(2-amino-3,5-dibrom-benzyl)amino]cyclohexanol; Mucosolvan®, Thomae GmbH, Biberach a.R., Germany) improves the biosynthesis and secretion of surfactant by alveolar type II cells. Furthermore, ambroxol is concentrated in the lung tissue and may also have antioxidative and antiinflammatory properties.[10–13]

In order to test the ability of ambroxol to improve the clinical course of RDS a multicenter, randomized double-blind trial was performed.[14] This report describes pulmonary function after extubation to assess the efficacy of ambroxol in newborns with RDS.

[1]Department of Neonatology (Charité), Humboldt University, Berlin, Germany.

[2]Department of Pediatrics, Technical University, Dresden, Germany.

Grant sponsors: German Ministry for Research and Technology, Project Perinatal Lung; Grant number: 01 ZZ 9511; Grant sponsor: DFG; Grant numbers: SdmU60/1–1, Wa 758/1–4.

*Correspondence to: Dr. Gerd Schmalisch, Department of Neonatology (Charité), Humboldt University, Schumannstraße 20/21, D - 10098 Berlin, Germany. E-mail: gerd.schmalisch@charite.de

Received 13 March 1997; accepted 25 August 1998.

MATERIALS AND METHODS

Patient Management

The clinical trial[14] was conducted concurrently in the neonatal units of Humboldt-University (Berlin) and the Technical University (Dresden). Entry was limited to very low birth weight infants requiring mechanical ventilation due to established RDS. Admission criteria were: 1) birth weight <1,500g and gestational age <34 weeks, 2) radiological findings consistent with RDS (gradation according to Bomsel[15]), 3) need for mechanical ventilation, and 4) $F_{I,0_2}$ >0.4 to maintain $P_{a,0_2}$ >6.5 kPa (50 mmHg). Exclusion criteria were: 1) positive blood cultures in umbilical cord blood samples at birth, 2) major malformations detected after enrollment (trisomies 13 and 18; cardiac malformations except ductus arteriosus persistens, gastroschises, and intestinal atresia), and/or 3) failure to complete study protocol.

The sample size calculation was based on the hypothesis that ambroxol treatment would lead to an earlier improvement than placebo in: 1) pulmonary gas exchange (indicated by $P_{a,O_2}/F_{I,O_2}$ ratio), 2) phospholipid profile in tracheal effluent as indicated by evidence of phosphatidylglycerol, 3) mean airway pressure during mechanical ventilation, and 4) lung mechanics after extubation as indicated by $V_T/ P_{es,max}$. Assuming a 40% improvement in these four endpoints, we estimated (type I error = 5%, type II error = 10%) that 102 newborns would be required in the two groups.[14] The patient recruitment was finished after 102 newborns surviving 28 days were enrolled. Patient characteristics of both treatment groups are shown in Table 1.

TABLE 1—Characteristics of Patients Surviving 28 Days[1]

	Ambroxol	Placebo	*P*
Number of patients	52	50	
Birth weight (g)	1,238 ± 203	1,207 ± 221	n.s.
Gestational age (weeks)	29.6 ± 1.6	29.3 ± 1.9	n.s.
Boys	31/52 (60%)	29/50 (58%)	n.s.
pH	7.23 ± 0.12	7.25 ± 0.09	n.s.
APGAR score (5 min) <5	5/52 (10%)	3/50 (6%)	n.s.
RDS stage on day 1			
I/II	20/52 (38%)	22/50 (44%)	
III/IV	32/52 (62%)	28/50 (56%)	n.s.
Duration of artificial ventilation (h) median (range)	178 (36–1,845)	246 (70–2,068)	<0.05

[1]n.s., not significant.

During the first 5 days of life each infant received 30 mg/kg ambroxol or saline daily. Vials of ambroxol or saline were supplied and randomized by Thomae GmbH. A blinded vial contained either 2 mL of saline or 15 mg ambroxol diluted in 2 mL saline. The total daily dose was divided into four individual doses of 7.5 mg/kg and given as an infusion over 5 min every 6 h.

Ethics

The clinical trial was reviewed and approved by the ethics committees of both hospitals, and informed consents were obtained from the parents.

Lung Function Testing

Measurements of ventilation and lung mechanics were performed after extubation on days 7, 14, 21, and 28. The infant was placed supine with the neck in neutral position. All infants were dry and clean, and LFT was performed about 30 min after feeding. No sedatives were used.

For bedside measurements, our self-constructed equipment utilized a flow-through technique (FTT).[16] The basic principle of FTT in combination with esophageal manometry is shown in Figure 1. The face mask is flushed by a constant background flow of gas (V'_{const}) to compensate for the apparatus dead space. We used the face chamber FC-100 (Siemens-Elema, Lund, Sweden) with a removable transparent lid and various sizes of latex cuffs to fit the faces of infants of different size.

A pneumotachometer (PNT 1) measured the airflow into the face mask, and a second PNT (PNT 2) measured the flow coming out. The difference of both flow signals (V'_{diff}) was the ventilation of the infant, provided both PNTs had identical calibration characteristics and there was no air leak. Using the FTT, air leaks of the face mask can be measured and expressed in percentages by $100 \cdot \overline{V'}_{diff}/\overline{V'}_{const}$, where $\overline{V'}_{diff}$ is the mean difference in

Abbreviations

C	Compliance
f	Respiratory frequency
F*	Optimal respiratory frequency
$F_{I,0_2}$	Inspiratory oxygen fraction
FTT	Flow-through technique
LFT	Lung function testing
P_{a,O_2}	Arterial oxygen tension
$P_{es,max}$	Magnitude of esophageal pressure changes
$P_{es}(t)$	Esophageal pressure with respect to time
PNT	Pneumotachometer
PTIF	Peak tidal inspiratory flow
R	Resistance
RDS	Respiratory distress syndrome
t_L	Time constant of the lung
V	Volume
V_D	Dead space
V_T	Tidal volume
V_T*	Optimal tidal volume
V'	Air flow
V'_A	Alveolar ventilation
V'_{const}	Constant background flow
V'_E	Minute ventilation
$V_T/P_{es,max}$	Ratio of tidal volume to maximal esophageal pressure changes
W	Work of breathing
W′	Power of breathing

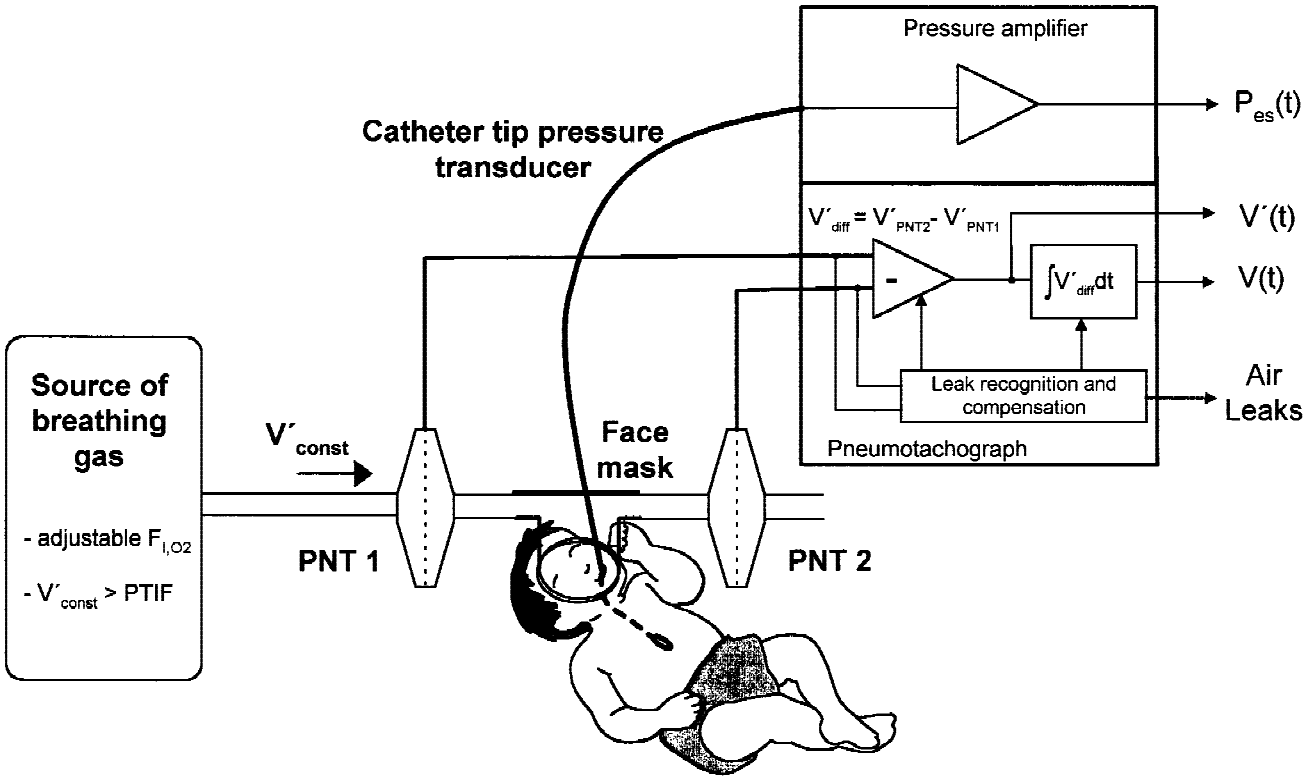

Fig. 1. Equipment for bedside lung function testing in preterm infants using the flow-through technique and esophageal pressure measurements. Both pneumotachometers (PNT 1, PNT 2) have the same calibration, so that the difference of both flow signals V'_{diff} (t) represents the inspiratory and expiratory flow of the infant independent of the background flow V'_{const}.

flow averaged over a defined number of breathing cycles. The background flow was always higher than the peak tidal inspiratory flow (PTIF),[17] and the F_{I,O_2} was adjusted according to therapeutic requirements. The volume calibration was carried out with the same background flow used during the performance of the LFT.

For assessment of lung mechanics, esophageal pressure changes were measured using a catheter tip pressure transducer (Messgeraete-Werk Zwoenitz, Zwoenitz, Germany). The catheter diameter was 1.4 mm and the pressure transducer at the tip was 1.7 mm in diameter. The catheter was passed through a specially prepared opening in the chamber and was advanced via the oral cavity to the stomach. Once the newborn was asleep, the lid of the chamber was closed. The position of the catheter tip was guided by the simultaneously monitored volume and pressure signals. Rise of pressure during inspiration indicated placement in the stomach. To optimize esophageal pressure measurements, the catheter was pulled back to the region of maximal esophageal pressure changes and minimal cardiac effects on the esophageal pressure signals.

Following satisfactory placement of the esophageal catheter, the infants were allowed to adjust for 5–20 min. During this adaptation period a steady state was reached, characterized by low respiratory frequency and a regular breathing pattern.

Artifact-free breathing cycles (5–10) were evaluated, and tidal volume (V_T), respiratory frequency (f), and maximal esophageal pressure changes ($P_{es,max}$) were determined. From these parameters, minute ventilation V'_E and ratio of V_T to $P_{es,max}$ ($V_T/P_{es,max}$) were calculated. Instead of measuring pulmonary compliance (C) and resistance (R) according to Mead-Whittenberger analysis,[18] the parameter $V_T/P_{es,max}$ was determined; it is independent of any assumptions about lung mechanics (e.g., assumption of a linear one-compartment R-C model), and it describes primarily the elastic properties of the lung.

At this age pulmonary parameters can be compared only when we consider the infants' maturational and developmental stage. Therefore, we developed percentile curves[19] for the measured parameters at different body weights (Fig. 2). In this study the results of LFT were presented as actually measured, and according to their location on the percentile curves.

Statistical Methods

Contingency tables were used to analyze qualitative parameters. The statistical significance of changes was evaluated using the Chi-square test or Fisher's exact test (one-tailed), whenever cell-frequencies in the 2 × 2 tables were smaller than 5. Means, standard deviations, and

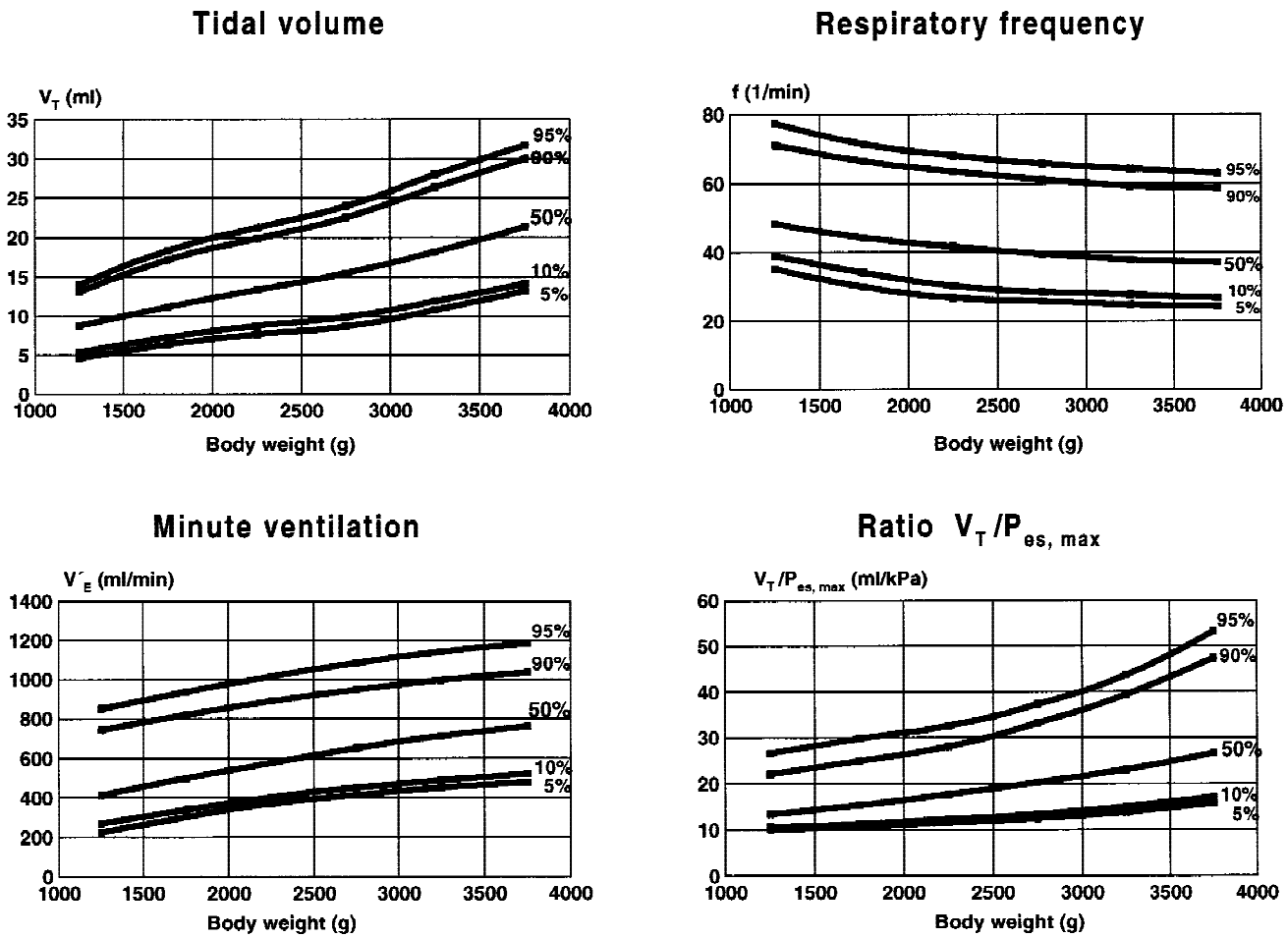

Fig. 2. Percentile curves[19] of pulmonary parameters in healthy newborns related to body weight.

standard errors of the mean (SEM) were calculated, and they were compared by two-tailed Student's t-test, provided the data were distributed normally. For nonparametric data (e.g., duration of mechanical ventilation), differences between groups were evaluated using rank tests (Wilcoxon, Mann-Whitney). A level of statistical significance of $P < 0.05$ was accepted. For statistical evaluation, the software STATGRAPHICS (Manugistics, Inc.) was used.

RESULTS

Preterm infants (102) were randomly assigned to receive ambroxol (52) or placebo (50). Table 2 shows the characteristics of extubated patients in whom lung function was measured, and there were no significant differences between both treatment groups. Extubation during the first 4 weeks of life occurred in 42/52 (81%) in the ambroxol group and 36/50 (72%) in the placebo group; on day 7, 24/42 (57%) infants in the ambroxol group and 16/36 (44%) in the placebo group were extubated.

The absolute and percentile tidal volumes (Fig. 3) in both groups were nearly identical, and V_T remained nearly constant around the 50th percentile for the duration of the observation period. The differences in respiratory frequency were also not significant (Fig. 4). Transformation into percentiles shows that the respiratory frequencies of both groups were slightly above the 50th

TABLE 2—Characteristics of Extubated Newborns on Whom Lung Function Testing Was Performed During the First 4 Weeks of Life[1]

	Ambroxol	Placebo	*P*
Number of extubated newborns during first 4 weeks of life	42/52 (81%)	36/50 (72%)	n.s.
Birth weight (g)	1,250 ± 199	1,273 ± 191	n.s.
Gestational age (weeks)	29.6 ± 1.6	29.9 ± 1.7	n.s.
Boys	26/42 (62%)	19/36 (53%)	n.s.
pH	7.22 ± 0.12	7.26 ± 0.08	n.s.
APGAR score (5 min) <5	5/42 (12%)	1/36 (3%)	n.s.
RDS stage on day 1			
I/II	20/42 (48%)	20/36 (56%)	
III/IV	22/42 (52%)	16/36 (44%)	n.s.
Duration of artificial ventilation (h) median (range)	160 (36–555)	182 (70–552)	n.s.

[1]n.s., not significant.

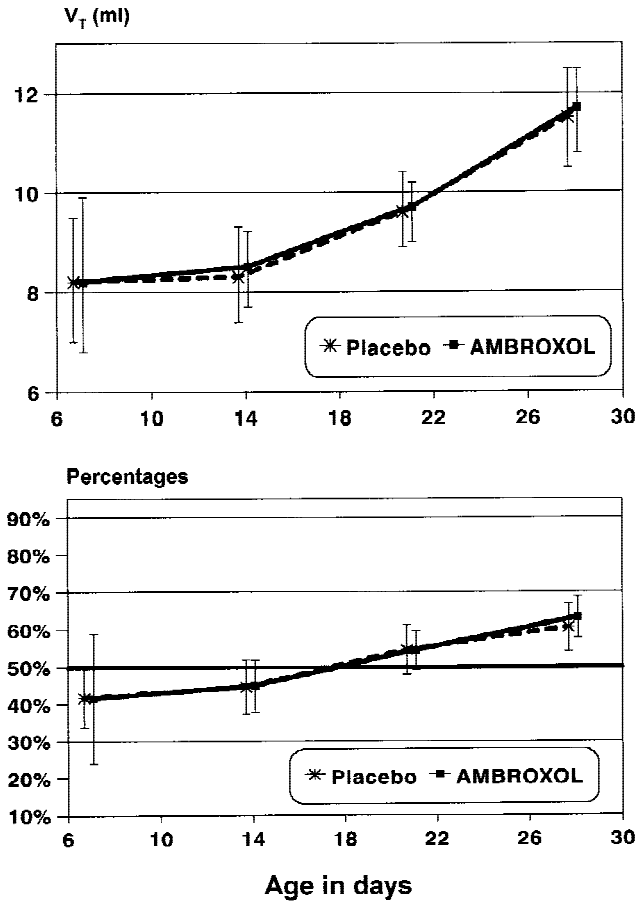

Fig. 3. Changes in absolute tidal volume (top) and percentiles (bottom) in ambroxol and placebo treatment groups (mean 2 SEM).

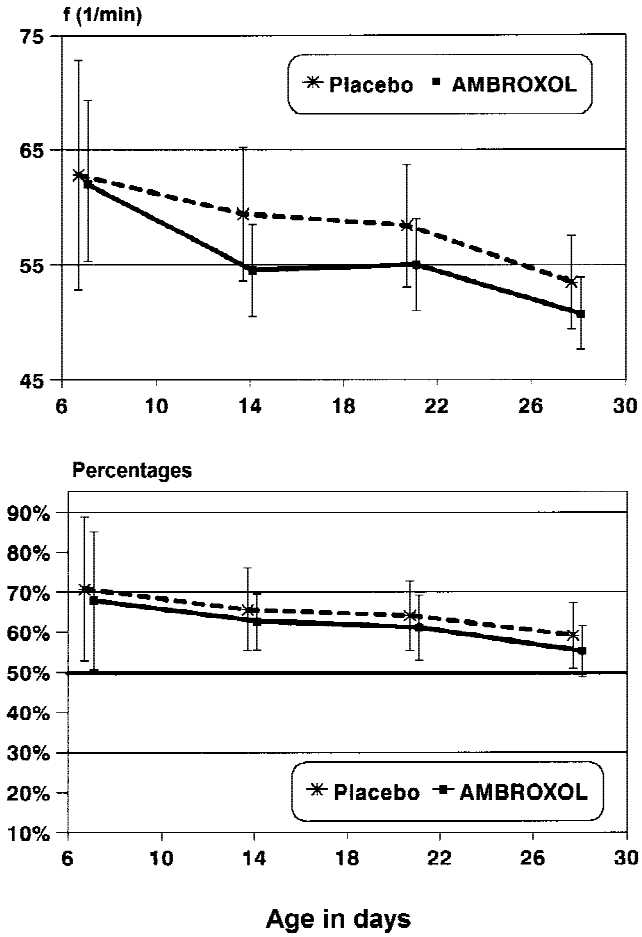

Fig. 4. Changes in absolute respiratory frequency and percentiles in ambroxol and placebo treatment groups (for abbreviations see Fig. 3).

percentile. No significant differences were noted in minute ventilation (Fig. 5).

In contrast to the ventilatory parameters, significant differences in the lung mechanic parameter $V_T/P_{es,max}$ (Fig. 6) were observed between the two treatment groups. For both groups, $V_T/P_{es,max}$ lay below the 10th percentile and normalization of the data showed significant differences in the two groups on day 7. In the ambroxol group, $V_T/ P_{es,max}$ reached the 10th percentile on day 10, i.e., significantly earlier ($P < 0.05$) than in the placebo group (day 23). On day 28, the lung mechanics in both groups had normalized, and the values for both groups were no longer significantly different.

DISCUSSION

The aim of this clinical trial was to test whether ambroxol could improve the course of RDS in preterm newborns and bring about a significant improvement of all endpoints.[14] This trial was carried out in the former German Democratic Republic at a time when surfactant was not available. Therefore, the observed pharmacological effect is not influenced by surfactant replacement.

In contrast to the clinical results, the effect of ambroxol treatment on the development of pulmonary functional parameters is more difficult, because:

1) On day 7 only half of the infants in both groups were extubated, but the other half were also included in the subsequent lung function study;
2) The patients who were extubated early represent a relatively healthy group of patients because most of the severely ill infants in both groups were ventilated throughout the neonatal period;
3) We cannot answer whether there was a therapeutic effect of ambroxol on lung function in very sick ventilated infants.

Despite these limitations, a significant improvement of lung mechanics could be demonstrated in the ambroxol-treated group, although the therapeutic effect was modest and could be measured only in the first weeks after ex-

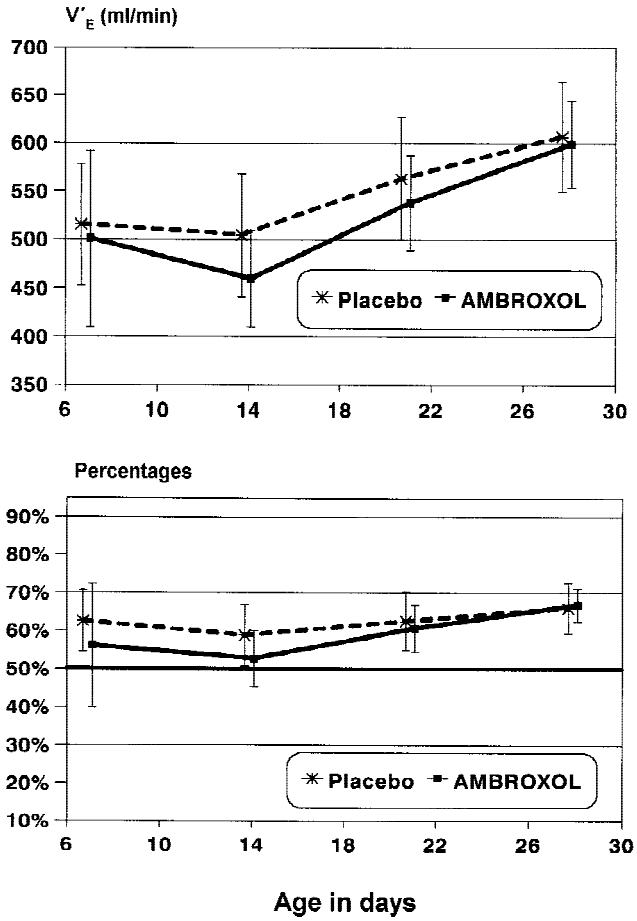

Fig. 5. Changes in minute ventilation (absolute values and percentiles) in ambroxol and placebo treatment groups (for abbreviations see Fig. 3).

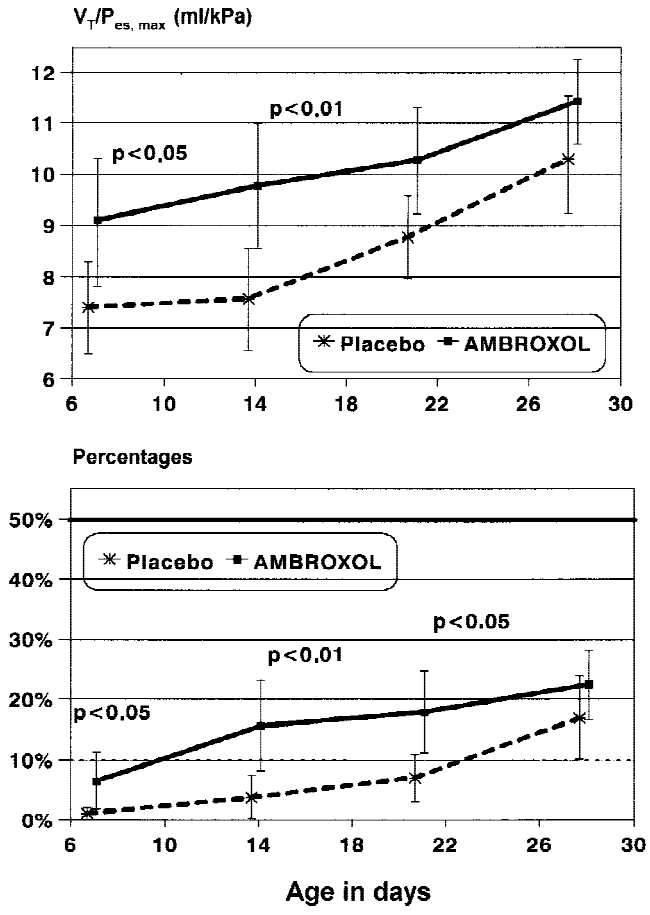

Fig. 6. Changes in V_T/ $P_{es,max}$ (absolute values and percentiles) in ambroxol and placebo treatment groups (for abbreviations see Fig. 3).

tubation. Ambroxol therapy did not affect ventilatory parameters.

We can explain the small differences between the measured ventilatory parameters in both treatment groups by an examination of the energetics of breathing, based on the calculation of power expenditure for breathing, assuming sinusoidal respiratory signals and a simple one-compartment model of the lung (see Appendix). This model was used by Cook et al.[20] to investigate the relationship between lung mechanics and respiratory frequency during tidal breathing in newborns. This model can also be used to explain the relationship between ventilatory patterns and lung mechanics.

According to the assumptions described in the appendix, there is an optimal respiratory pattern (V_T*, f*) for a given compliance (C), resistance (R), and dead space (V_D), which produces the necessary alveolar ventilation (V'_A) with minimal power expenditure for breathing (Fig. 7). As shown in Figure 7, changes in lung mechanics influence tidal volume and respiratory frequency differently. When C increases, the increase of V_T* is negligible, and the changes in f* are minimal and measurable only for low values of C. If C is higher than 15 mL/kPa, changes in lung compliance will not be detectable by changes in the ventilatory parameters, when we consider the high variability of respiratory parameters at this age. This may explain the results of LFT, because in this study LFT was performed only in spontaneously breathing infants who were recovering from RDS and displayed near-normal lung mechanics.

The use of percentile curves simplified the interpretation of the measured pulmonary parameters. Despite the rapid increase of V_T, the transformation of V_T to percentiles of healthy newborns revealed that in both groups the tidal volume lay around the 50th percentile during the whole period of observation (Fig. 3). This suggests that in both groups there is a normal physiologic increase in V_T which is not the result of any therapeutic intervention. Because all ventilatory parameters lie within the normal range, even immediately after extubation, a measurable therapeutic effect of ambroxol on ventilatory parameters cannot be expected.

The lung mechanics parameter V_T/ $P_{es,max}$ was the only one which reflected the severity of respiratory dis-

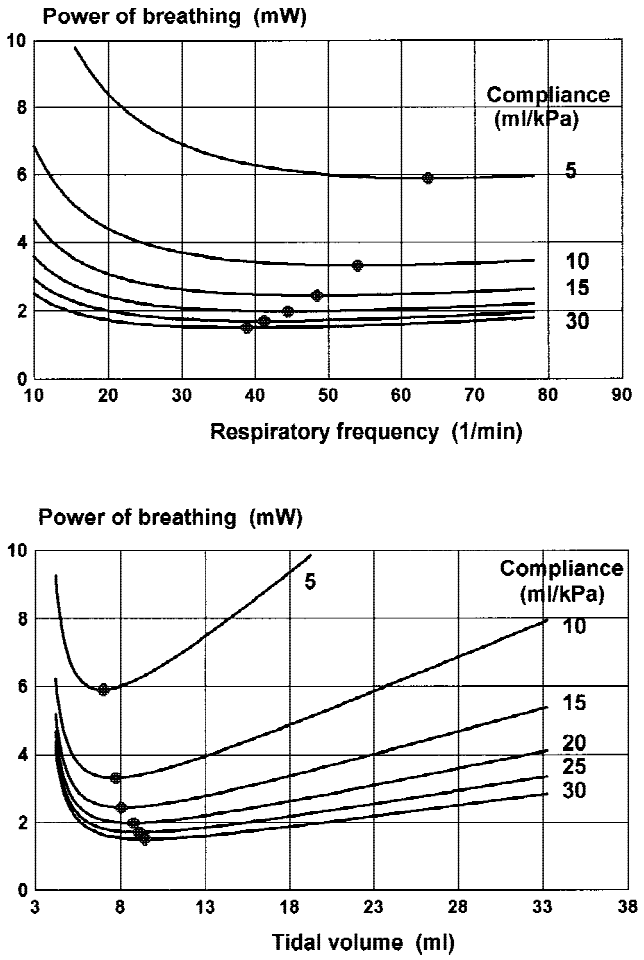

Fig. 7. Relationship between power of breathing and respiratory frequency (top) and tidal volume (bottom) at different values for pulmonary compliance. Alveolar ventilation (V'_A = 250 mL/min) and dead space (V_D = 5 mL) were kept constant. The calculations are based on a one-compartment model and a sinusoidal respiratory signal (see Appendix). Optimal respiratory energetics are indicated by circles.

ease during the first days of life. This confirms the clinical experience that measurements of ventilatory parameters in newborns with respiratory disease are of limited diagnostic value, and that it is necessary to investigate lung mechanics at this age.[21–23]

Despite the well-known methodological problems of esophageal manometry,[24–26] measurements of esophageal pressure are a suitable and, for clinical decisions, a reliable method for the assessment of lung mechanics, especially in preterm infants.[27] Combination of esophageal manometry with the FTT improves the usefulness of this technique considerably. An important advantage of the FTT is the possibility to adjust the F_{I,O_2} and to continuously measure air leaks of the mask. In contrast to currently used equipment for LFT, measurements are not time-limited because the apparatus dead space is eliminated by the continuous background gas flow. The FTT allows the investigator to adjust the adaptation period after placement of the esophageal catheter until a steady state in the signals is reached.

After insertion of the esophageal catheter and adjustment of the face mask, volume and pressure signals can be displayed simultaneously. This helps in placement of the esophageal catheter in the area of maximal esophageal pressure swings and minimal interference by cardiac pulsations. The main disadvantage of the FTT is that occlusion tests which would help to validate the esophageal pressure measurements cannot be performed.[25] Therefore, in this study $P_{es}(t)$ is only a measure of respiratory efforts, and the maximal peak-to-peak pressure differences along the esophagus were used to standardize the measurements.

The parameter $V_T/P_{es,max}$ can be calculated quite simply, regardless of the status of lung mechanics, even in infants with pulmonary inhomogeneities. However, $V_T/P_{es,max}$ does not allow a differentiation between changes in the viscous and elastic properties of the lung, and we cannot describe the therapeutic effects of ambroxol therapy on lung mechanics in more detail. Cook et al.[20] have already shown that about 70% of the pulmonary work of breathing is due to the elastic resistance of the lung, and this is increased in infants with RDS. Therefore, we assume that the observed improvement in lung mechanics is caused by an improvement in the elastic properties of the lung. This conclusion would support the hypothesis that ambroxol accelerates recovery of lung function caused by surfactant deficiency, by improving production of surfactant, or by its antioxidative and antiinflammatory properties.

Up to now the effects of ambroxol on lung development in newborns with RDS are unknown in detail. Investigations by Sun et al.[7] in preterm rabbits did not show a large effect of prenatal ambroxol treatment on lung maturation. In the ambroxol group, they found a modest increase in the lung tissue pool that is saturated with phosphatidylcholine. There were no significant improvements in lung mechanics as evaluated by static pressure-volume curves and only a trend toward increased lung volumes.[7] In our clinical trial, ambroxol was given postnatally to newborns with established RDS, and we found a modest (but significant) improvement in lung mechanics immediately after extubation. The therapeutic benefits of ambroxol treatment on lung mechanics were not of the same magnitude as the effects achieved by surfactant replacement.

APPENDIX

A simple concept to explain the relationship between ventilatory parameters (V_T, f, V'_E) and lung mechanics is based on the minimization of the power of breathing. It is assumed that during tidal breathing, depth and frequency

of breathing cycles are adjusted in such a way that alveolar ventilation

$$V'_A = f \cdot (V_T - V_D) \quad (1)$$

ensures adequate alveolar gas exchange with a minimal power expenditure for breathing. V_D is the physiologic dead space which describes the intrapulmonary gas volume not involved in alveolar gas exchange. Assuming a linear one-compartment model of the lung

$$R \cdot C \cdot V'(t) + V(t) = -C \cdot P_{es}(t) \quad (2)$$

and sinusoidal transpulmonary pressure changes $P_{trans}(t)$ (measured by esophageal pressure $P_{es}(t)$), the power of breathing (W') can be expressed by

$$W'(f) = \frac{V_T^2 \cdot f}{2 \cdot C}\left(1 + \frac{\pi^2}{2} R \cdot C \cdot f\right). \quad (3)$$

This equation is well-known as the simplified formula of Otis, Fenn and Rahn.[20] If alveolar ventilation (V'_A), dead space (V_D), and lung mechanics (R, C) are known, the relationship between the power of breathing and the ventilatory parameters (V_T, f) can be calculated by combination of Eq. 1 and Eq. 3:

$$W'(f) = \frac{(V'_A + V_D \cdot f)^2}{2 \cdot C \cdot f}\left(1 + \frac{\pi^2}{2} R \cdot C \cdot f\right) \quad (4)$$

$$W'(V_T) = \frac{V_T^2 \cdot V'_A}{2 \cdot C \cdot (V_T - V_D)}\left(1 + \frac{\pi^2}{2} R \cdot C \cdot \frac{V'_A}{(V_T - V_D)}\right). \quad (5)$$

Both functions $W'(f)$ and $W'(V_T)$ are convex (see Fig. 7), and the minima characterize the energetic optimum. The energetic optimal respiratory frequency (f*) and tidal volume (V_T*) can be determined by solving the equations

$$\frac{\partial W'(f)}{\partial f} = 0 \quad \text{and} \quad \frac{\partial W'(V_T)}{\partial V_T} = 0. \quad (6)$$

With the pulmonary time constant $t_L = R \cdot C$, the solutions of Eq. 6 are:

$$f^* = \frac{1}{2 \cdot \pi^2 \cdot t_L}\left(\sqrt{1 + \frac{\pi^2 \cdot t_L \cdot V'_A}{4 \cdot V_D}} - 1\right)$$

$$V_T^* = V_D\left(1.5 + 0.5\sqrt{1 + \frac{\pi^2 \cdot t_L \cdot V'_A}{4 \cdot V_D}}\right). \quad (7)$$

These two formulas show that f* and V_T* depend only on three parameters: pulmonary time constant (t_L), alveolar ventilation (V'_A), and dead space (V_D). Using this model, the effect of changes in the lung mechanics on the respiratory pattern can be approximated.

REFERENCES

1. Avery S, Mead J. Surface properties in relation to atelectasis and hyaline membrane disease. Am J Dis Child 1959;97:517–523.
2. Jobe AH. Pulmonary surfactant therapy. N Engl J Med 1993;328: 861–868.
3. Mercier CE, Soll RF, Lucey JF. Clinical trials of natural surfactant extract in respiratory distress syndrome. Clin Perinatol 1991;12: 261–267.
4. Gortner L. Natural surfactant for neonatal respiratory distress syndrome in very premature infants: a 1992 update. J Perinat Med 1992;20:409–419.
5. Halliday HL. Surfactant replacement therapy. Pediatr Pulmonol [Suppl] 1995;11:96–97.
6. Wauer RR, Schmalisch G, Hammer H, Buttenberg S, Weigel H, Huth M. Ambroxol for prevention and treatment of hyaline membrane disease. Eur Respir J [Suppl] 1989;3:57–65.
7. Sun B, Rider E, Ikegami M, Jobe A. Antenatal ambroxol effects on surfactant pool size and postnatal lung function in preterm ventilated rabbits. Biol Neonate 1992;62:55–62.
8. Hallman M, Bry K, Hoppu K, Lappi M, Pohjavuori M. Inositol supplementation in premature infants with respiratory distress syndrome. N Engl J Med 1992;326:1233–1239.
9. von Seefeld H, Weiss JM, Eberhardt H. Stimulation of lung maturity: investigation of ambroxol in various animal models. Acta Physiol Hung 1985;65:305–312.
10. Kimya Y, Kucukkomurcu S, Ozan H, Uncu G. Antenatal ambroxol usage in the prevention of infant respiratory distress syndrome. Beneficial and adverse effects. Clin Exp Obstet Gynecol 1995;22:204–211.
11. Nowak D, Antczak A, Pietras T, Bialasiewicz P, Krol M. Protective effect of ambroxol against heat- and hydrogen peroxide-induced damage to lung lipids in mice. Eur Respir J 1994;7:1629–1634.
12. Lapenna D, de Gioia S, Ciofani G, Cuccurullo F. Ambroxol is a scavenger of hypochlorous acid and monochloramine. Pharmacology 1994;49:132–135.
13. Bianchi M, Mantovani A, Erroi A, Dinarello CA, Ghezzi P. Ambroxol inhibits interleukin 1 and tumor necrosis factor production in human mononuclear cells. Agents Actions 1990;31:275–279.
14. Wauer RR, Schmalisch G, Bohme B, Arand J, Lehmann D. Randomized double blind trial of ambroxol for the treatment of respiratory distress syndrome. Eur J Pediatr 1992;151:357–363.
15. Bomsel F. Contribution a l'étude radiologique de la maladie des membranes hyalines. A propos de 110 cas. J Radiol Electrol 1979; 51:259–268.
16. Schmalisch G, Wauer RR, Beier E, Wunsche A. Measuring equipment for bedside diagnosis of respiratory function in spontaneously breathing newborn infants. Biomed Tech (Berlin) 1991;36: 44–50.
17. Schmalisch G, Wauer RR. Adjusting background flow in measuring ventilation of newborn infants and infants using the flow-through technique. Pneumologie 1995;49:461–465.

18. Stocks J, Sly PD, Tepper RS, Morgan WJ. Infant respiratory function testing. New York: Wiley-Liss; 1997. p 1–577.
19. Schmalisch G, Grubba K, Wauer RR. Computer aided interpretation of pulmonary parameters in newborn infants. Med Biol Eng Comput 1996;34:160–164.
20. Cook CD, Sutherland JM, Segal S, Cherry RB, Mead J, McIlroy MB, Smith CA. Studies of respiratory physiology in the newborn infant. III. Measurements of mechanics of respiration. J Clin Invest 1957;36:440–448.
21. Stocks J, Dezateux CA, Jackson EA, Hoo AF, Costeloe KL, Wade AM. Analysis of tidal breathing parameters in infancy: how variable is tptef:te. Am J Respir Crit Care Med 1994;150:1347–1354.
22. Mikkilineni S, England S. On tidal expiratory flow measurements in infants. Pediatr Pulmonol 1994;18:71–72.
23. Seddon PC, Davis GM, Coates AL. Do tidal expiratory flow patterns reflect lung mechanics in infants? Am J Respir Crit Care Med 1996;153:1248–1252.
24. Heaf DP, Turner H, Stocks J, Helms P. The accuracy of esophageal pressure measurements in convalescent and sick intubated infants. Pediatr Pulmonol 1986;2:5–8.
25. Coates AL, Stocks J. Esophageal pressure manometry in human infants. Pediatr Pulmonol 1991;11:350–360.
26. LeSouef PN, Lopes JM, England SJ, Bryan MH, Bryan AC. Influence of chest wall distortion on esophageal pressure. J Appl Physiol. 1983;55:353–358.
27. Silva Neto G, Gerhardt TO, Claure N, Duara S, Bancalari E. Influence of chest wall distortion and esophageal catheter position on esophageal manometry in preterm infants. Pediatr Res 1995; 37:617–622.