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Effect of early Ambroxol treatment on lung functions in mechanically ventilated preterm newborns who subsequently developed a bronchopulmonary dysplasia (BPD)[1]

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In a randomized trial in 102 preterm newborns with respiratory distress syndrome (RDS) it has been shown that early Ambroxol treatment (30 mg kg^{-1} over the first 5 days) significantly reduces the incidence of RDS-associated complications [bronchopulmonary dysplasia (BPD), intraventricular haemorrhage, post-natal acquired pneumonia]. The aim of the present analysis was to investigate the effect of Ambroxol treatment on lung function in newborns who developed BPD.

Respiratory function testing (RFT) was performed immediately after extubation and at day 28. Tidal volume (V_T) and respiratory frequency (f) were measured during tidal breathing using the deadspace free flow-through technique. The lung mechanic parameter $V_T/maxP_{es}$ was determined by measuring the maximal oesophageal pressure changes, $maxP_{es}$, with a catheter tip pressure transducer.

In the placebo group 36/50 infants were extubated within the first 28 days of life and 13/36 (36%) developed BPD. In the Ambroxol group 44/52 were extubated and 9/44 (20%) developed BPD. After extubation, RFT showed (i) no statistically significant difference in the ventilatory parameters of either treatment group, (ii) improved ($P<0{\cdot}05$) lung mechanics ($V_T/maxP_{es}$) in Ambroxol group compared to controls ($9{\cdot}4\pm2{\cdot}7$ ml kPa^{-1} *vs.* $8{\cdot}1\pm2{\cdot}6$ ml kPa^{-1}) and (iii) no statistically significant difference in lung function between infants with and without BPD. At day 28 we found (i) no effect of early Ambroxol treatment on lung functions, (ii) significantly ($P<0{\cdot}05$) higher f ($58{\cdot}5\pm11{\cdot}7$ min^{-1} *vs.* $49{\cdot}7\pm10{\cdot}1$ min^{-1}) and significantly ($P<0{\cdot}01$) lower V_T ($9{\cdot}6\pm1{\cdot}9$ ml *vs.* $12{\cdot}3\pm2{\cdot}7$ ml) and $V_T/maxP_{es}$ ($8{\cdot}9\pm2{\cdot}6$ ml kPa^{-1} *vs.* $12{\cdot}0\pm2{\cdot}9$ ml kPa^{-1}) in infants with BPD compared to infants without and (iii) these differences are not influenced by early Ambroxol treatment.

If the process of BPD development is induced, early Ambroxol treatment has no influence on impaired lung function at day 28.

Key words: respiratory distress syndrome; surfactant deficiency; Ambroxol; lung function testing; newborns.

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Introduction

Ambroxol, a metabolite of bromhexine with a high affinity for lung tissue (1), is well established in the treatment of respiratory disorders of different aetiology. Ambroxol's most important pharmacological activity is mucokinetic and secretolytic, it stimulates synthesis and release of surfactant from type II pneumocytes, and has anti-oxidative and anti-inflammatory effects (2–4). The anti-oxidative function of Ambroxol has been studied in detail in *in vitro* investigations by Nowak *et al.* (5) in cell-free suspensions, and by Gillissen *et al.* (6) using mononuclear and polymorphonuclear cells.

A large number of preclinical and clinical studies have been performed to investigate the influence of prenatal or postnatal Ambroxol treatment on lung maturation [overview in (1)]. The results from these studies, however, are inconsistent with regard to the effect of Ambroxol on lung function in the developing lung (7,8).

In our previously published clinical trial (9) in preterm newborns <1500 g, the effect of early Ambroxol treatment on the course of the respiratory distress syndrome (RDS)

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was investigated in a randomized study. The main results of this study were that at day 28 the degree of respiratory support in the Ambroxol group was reduced and the incidence of bronchopulmonary dysplasia (BPD), intraventricular haemorrhage (IVH) and postnatal acquired pneumonia was significantly lower as compared to the control group. No adverse events were seen which could be attributed to the Ambroxol treatment.

In ventilated very low birth weight (VLBW) newborns, the BPD remains the most common pulmonary sequelae in survivors with an incidence of 20–50% depending on the definition used for characterization of BPD (10). The main factors contributing to the development of BPD are structural and functional immaturity (11), volotrauma (12), hyperoxia (13) and infections (14).

In view of its known qualities it was anticipated that early Ambroxol treatment would reduce the incidence of BPD at day 28 and also the degree of the lung function disorders in infants who developed BPD. Therefore, the aim of the present analysis was to investigate the effect of the early Ambroxol treatment on lung function by means of respiratory function testing (RFT) immediately after extubation and at day 28 in infants who fulfilled criteria of BPD (15).

Materials and methods

The clinical trial was a double-blind parallel group comparison, carried out in the neonatal units of the Humboldt University, Berlin, and the Technical University, Dresden. The clinical trial was reviewed and approved by the Ethics Committees of both hospitals, and informed consent was obtained from parents before randomization.

PATIENT MANAGEMENT

According to the estimated sample size (9), 102 preterm newborns with RDS who had survived day 28 were enrolled in the study. All newborns required mechanical ventilation during the first days of life because of severe respiratory insufficiency. Admission criteria were: (i) birthweight <1500 g and a gestational age of $<$ 34 weeks; (ii) radiological findings consistent with RDS (grading according to Bomsel (16)); (iii) a need for mechanical ventilation and (iv) a need for $FiO_2 > 0{\cdot}4$ to maintain a $PaO_2 > 6{\cdot}5$ kPa (50 mmHg). Exclusion criteria were (i) positive blood cultures in the umbilical cord blood samples at birth; (ii) major malformations detected after enrolment and (iii) incomplete study protocol. In accordance with the randomized assignment, 52 preterm infants received 30 mg kg^{-1} bodyweight Ambroxol (Mucosolvan, Dr Karl Thomae Ltd., Biberach) per day during the first 5 days of life and 50 infants received placebo (physiological saline). 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^{-1} and was given as infusion over 5 min every 6 h. No other drugs to improve gas exchange were administered.

RESPIRATORY FUNCTION TESTING

An important consideration in RFT in clinical trials is the choice of suitable tests and devices. In newborns the non-invasive single-breath occlusion technique is commonly used for measurements of lung mechanics. However, this test depends on several requirements (17) e.g. description of lung mechanics using a one compartment model, sufficient Herring–Breuer reflex, sufficient occlusion time for intra-alveolar pressure equilibration which cannot be taken for granted in the enrolled patients. To ensure data inclusion, a robust method of data capture was used based on a combination of flow-though technique (FTT) and oesophageal manometry.

Measurements of ventilation and lung mechanics were performed at the bedside during tidal breathing as described previously (18) using custom-made equipment. We used a face chamber (FC-100, Siemens-Elema, Sweden) with latex cuffs that could be altered in line with the body weight for optimal adaptation on the face. The face chamber was continuously rinsed thoroughly with a background flow (V'_{const}). This almost completely eliminated the apparatus dead space and enabled long-term measurements to be taken, even in oxygen-dependent newborns. Flows in and out of the chamber were measured by two screen pneumotachographs (Jaeger, Würzburg, Germany) and the infant's air flow was the difference between the two signals.

To assess lung mechanics, oesophageal pressure changes were measured using a catheter tip pressure transducer (Messgeraete Werk Zwoentiz, Germany) with a catheter diameter of 1·4 mm and a pressure transducer diameter of 1·7 mm at the tip. The catheter was placed in the lower third of the oesophagus in the area of the largest pressure changes. The placement was guided by the volume and pressure signals which were monitored simultaneously. The catheter was passed through the oral cavity downwards to the stomach. A rise in pressure on inspiration confirmed appropriate placement (Fig. 1). The catheter was then drawn back into the oesophagus up to the region of maximal pressure changes and minimal disturbances (e.g. heart activity). The infant was placed supine with the neck in the natural position. They were kept dry and clean and no sedatives were used. RFT was performed about 30 min after feeding. The FiO_2 of the background flow was adjusted in accordance with therapeutic requirements. The expiratory background flow was always higher than the peak tidal inspiratory flow (19). Calibration of the volume was carried out with the breathing gas used for the measurements.

Following optimal placement of the oesophageal catheter, the infants were allowed to adapt for 5–20 min. The adaptation period is commonly characterized by an initially high variability of the signals and high respiratory frequency. During this adaptation period the respiratory frequency decreases and steady state is reached, charcterized by low respiratory frequency and a more regular breathing pattern.

Depending on the variability of signals, 5–10 artefact-free breathing cycles were evaluated and tidal volume (V_T), respiratory frequency (f) and maximal oesophageal pressure

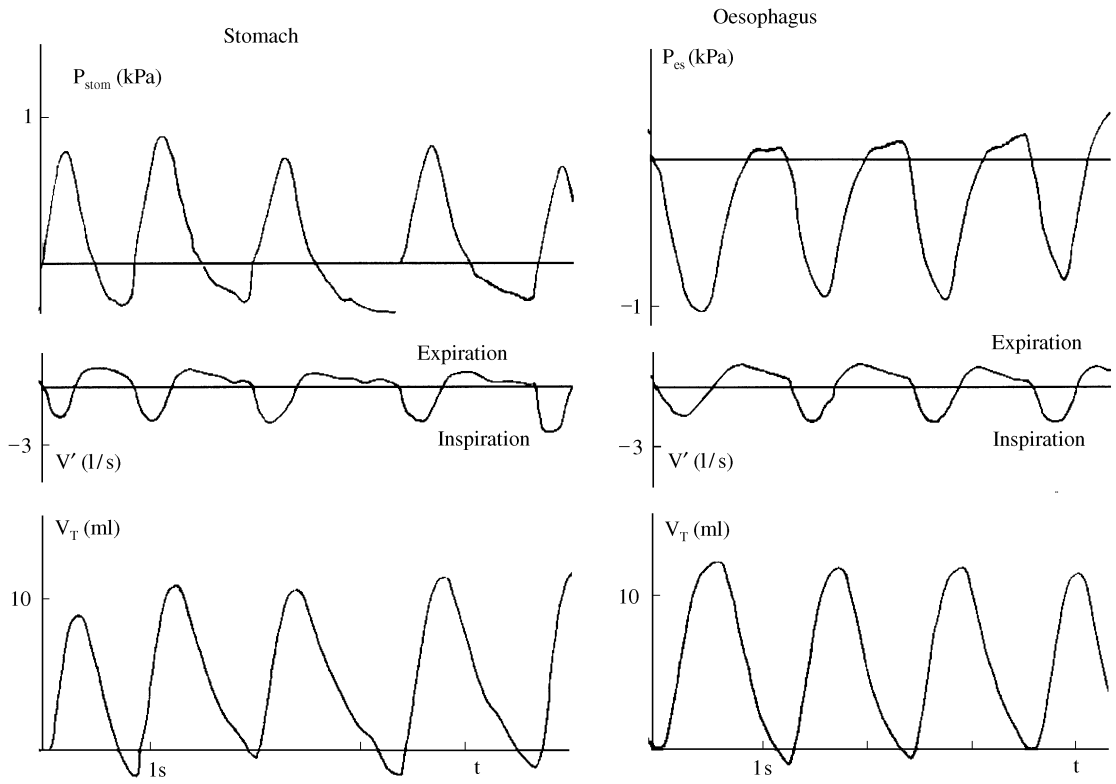

FIG. 1. Course of oesophageal pressure changes (top), air flow rate (middle) and volume (bottom) by placement of the catheter tip transducer in the stomach (on the left, visible by the rise in pressure at beginning of inspiration), and in the lower third of the oesophagus (on the right) with a clear decrease in pressure on inspiration.

changes ($maxP_{es}$) were determined to calculated the lung mechanic parameter ($V_T/maxP_{es}$). This parameter mainly describes the elastic properties of the lung and is independent from any assumption about lung mechanics (e.g. linear one-compartment model) (18).

STATISTICAL METHODS

Contingency tables were used to analyse qualitative parameters. The statistical significance was evaluated using the chi squared test or the Fisher exact test (one-tailed) if the cell frequencies in the two by two tables were smaller than five.

For quantitative data, mean and standard deviation (SD) or standard error mean (SEM) were calculated and for their comparison the two-tailed Students *t*-test was used, provided the data were distributed parametrically. All pulmonary parameters are presented as arithmetic group means with SD in the text and as mean with 95% confidence intervals in the figures. For non-parametric data (e.g. duration of mechanical ventilation), median and range were used and the differences between groups were investigated using rank tests (Wilcoxon, Mann–Whitney). For statistical evaluation the software STATGRAPHICS (Vers. 3.0, Manugistics Inc., U.S.A.) was used. A level of statistical significance of $P<0{\cdot}05$ was accepted.

Results

In the placebo group, 36/50 (72%) infants could be extubated within the first 28 days of life and 13/36 (36%) developed BPD. Simultaneously in the Ambroxol group the number of extubated infants was 44/52 (81%), and 9/44 (20%) developed BPD. As shown in Table 1 in both treatment groups essential patient characteristics influencing the clinical outcome did not differ significantly between the infants with and without BPD, except for the lower gestational age and the higher duration of mechanical ventilation in placebo-treated infants who developed BPD.

RFT AFTER EXTUBATION

Immediately after extubation, respiratory frequency (Fig. 2) and tidal volume (Fig. 3) did not differ significantly between either treatment group and their BPD subgroups. However, in infants treated with Ambroxol, the lung mechanics were slightly improved (pooled data of BPD and non-BPD infants) and the quotient $V_T/maxP_{es}$ (Fig. 4) was significantly higher ($P<0{\cdot}05$) compared to infants treated with placebo. With regard to clinical outcome, in neither treatment group were there statistically significant differences in lung function between infants with and without BPD at day 28.

TABLE 1. Characteristics of the investigated patients of both treatment groups with and without BPD at day 28 (mean ± SD or median and range)

	Placebo group $n = 36$			Ambroxol group $n = 44$		
	without BPD	BPD	P	without BPD	BPD	P
Number of infants	23 (64%)	13 (36%)		35 (80%)	9 (20%)	
Boys	10 (44%)	9 (69%)	0·17	19 (54%)	8 (89%)	0·12
Gestational age (weeks)	30·4 ± 1·4	29·0 ± 1·8	**0·02**	29·6 ± 1·5	29·2 ± 2·1	0·50
Birthweight (g)	1318 ± 152	1194 ± 239	0·07	1251 ± 1211	1256 ± 169	0·95
Body weight at day of extubation (g)	1332 ± 241	1225 ± 247	0·21	1257 ± 208	1230 ± 164	0·51
Body weight at day 28 (g)	1557 ± 243	1378 ± 324	0·07	1474 ± 258	1399 ± 169	0·42
Duration of mechanical ventilation (h) median (range)	144 (70–524)	240 (159–552)	**0·002**	158 (35–555)	188 (43–400)	0·72

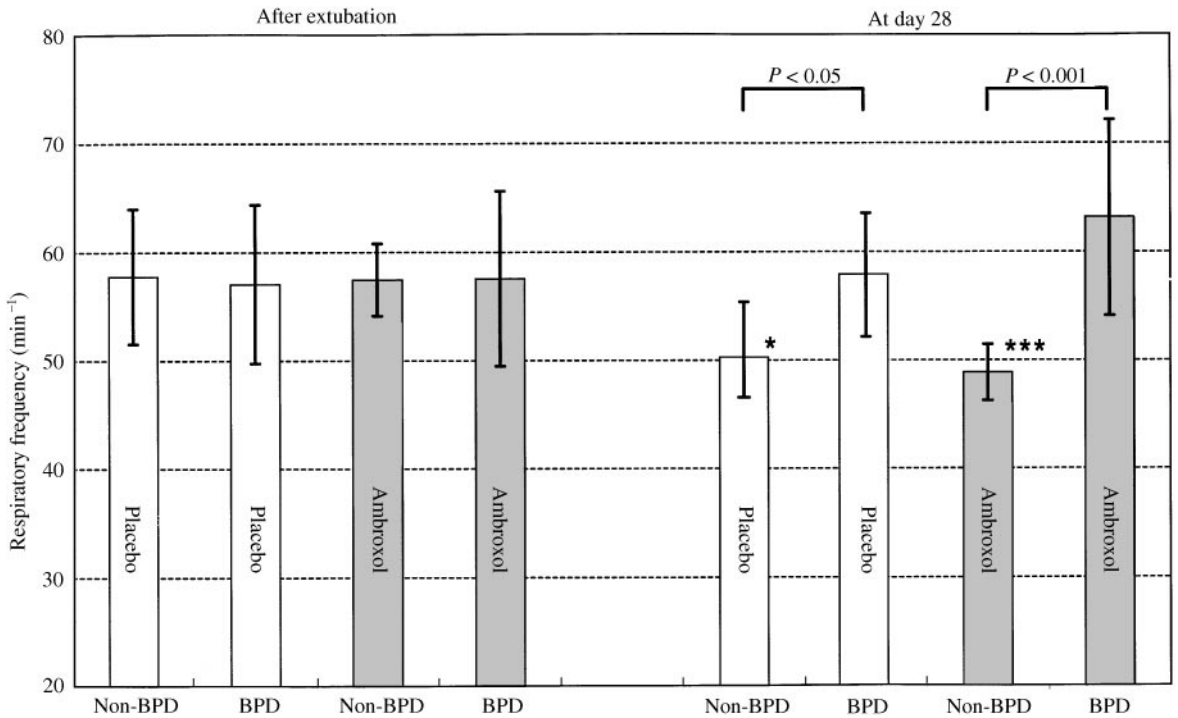

FIG. 2. Respiratory frequency after extubation (left) and at day 28 (right). The group means with the 95% confidence interval are presented. *$P < 0{\cdot}5$, ***($P < 0{\cdot}001$) = significant differences compared to the measurements after extubation.

RFT AT DAY 28

Early Ambroxol treatment had no influence on late respiratory function by day 28. Tidal volume (Fig. 3) was increased in all groups ($P < 0{\cdot}001$) compared to the initial measurements after extubation. The respiratory frequency was also significantly decreased ($P < 0{\cdot}05$) in infants without BPD, but unchanged in infants with BPD compared to the results after extubation. $V_T/maxP_{es}$ (Fig. 4) was only significantly improved ($P < 0{\cdot}001$) in infants without BPD.

In contrast to RFT after extubation, there were significant differences between infants with and without BPD at day 28 all measured parameters and these differences were similar to those in infants with and without Ambroxol treatment. Compared to infants without BPD at day 28 in both treatment groups, the infants with BPD had a significantly higher ($P < 0{\cdot}001$) respiratory frequency ($58{\cdot}5 \pm 11{\cdot}7$ min^{-1} *vs.* $49{\cdot}7 \pm 10{\cdot}1$ min^{-1}), a significantly lower ($P < 0{\cdot}001$) tidal volume ($9{\cdot}6 \pm 1{\cdot}9$ ml *vs.* $12{\cdot}3 \pm 2{\cdot}7$ ml) and $V_T/maxP_{es}$ ($8{\cdot}9 \pm 2{\cdot}6$ ml kPa^{-1} *vs.* $12{\cdot}0 \pm 2{\cdot}9$ ml kPa^{-1}).

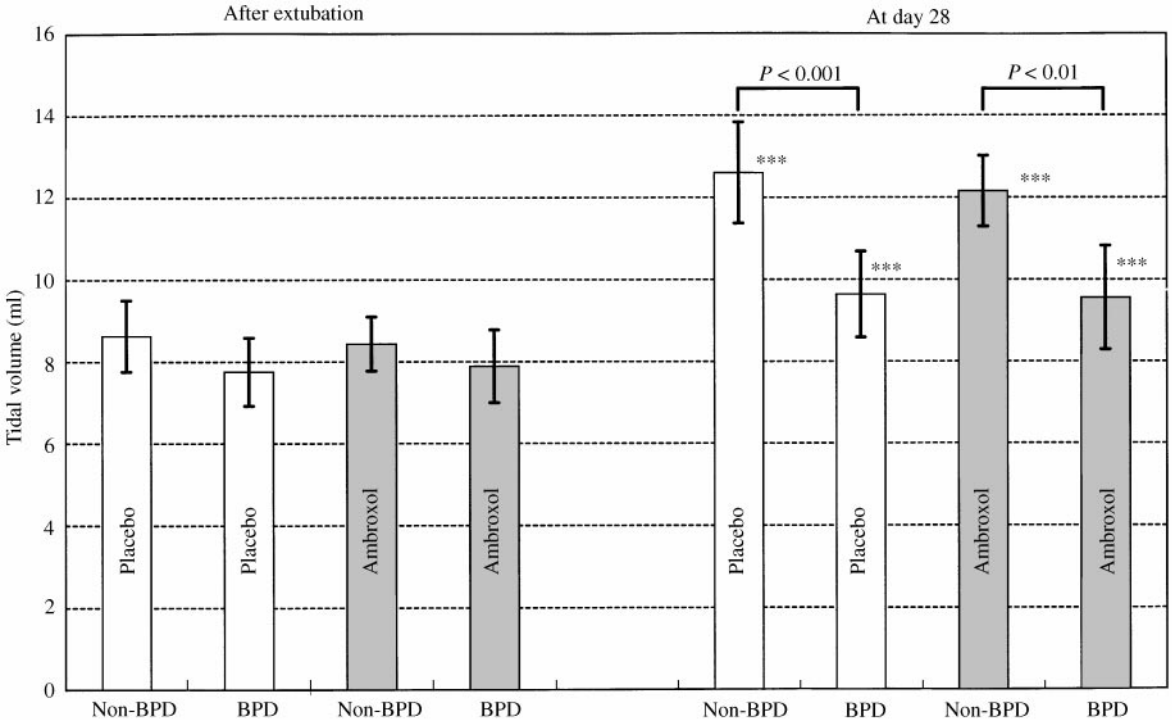

FIG. 3. Tidal volume after extubation (left) and at day 28 (right). The group means with the 95% confidence interval are presented. ***$P < 0{\cdot}001$ = significant differences compared to the measurements after extubation.

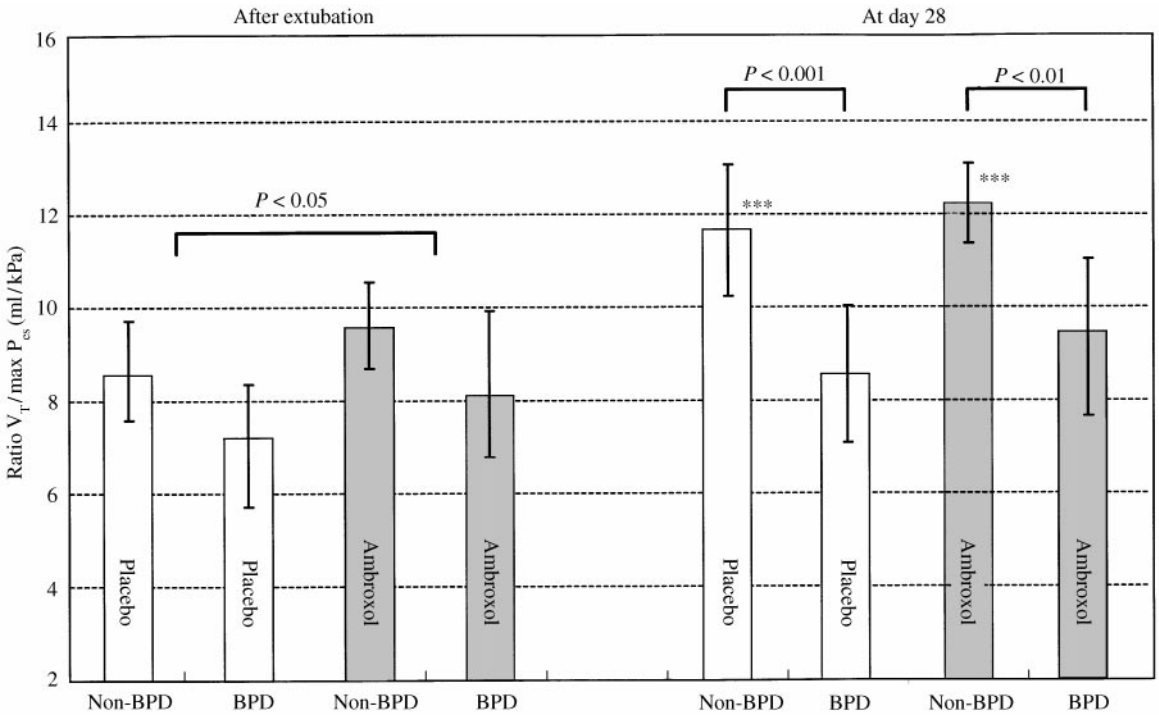

FIG. 4. The ratio $V_T/\text{max}P_{es}$ after extubation (left) and at day 28 (right). The group means with the 95% confidence interval are presented. ***$P < 0{\cdot}001$ = significant differences compared to the measurements after extubation.

Discussion

To investigate the effect of early Ambroxol treatment on lung function in BPD infants, we have re-evaluated data of our clinical trial (9) which was carried out in the former GDR at a time when surfactant therapy was not available and the pharmacological effect of the Ambroxol treatment was not influenced by surfactant replacement. This study is unique in the sense that in the future the effects of Ambroxol will probably never again be investigated in this group of untreated RDS patients since surfactant is considered standard treatment for neonatal RDS.

The method of RFT used in this clinical trial (9) is suitable to investigate lung function in VLBW infants (18). Immediately after extubation, we found that Ambroxol treatment had a moderate effect on lung mechanics, but that this was not detectable by means of ventilatory measurements. In a previous study (18) we showed that in preterm mechanically ventilated newborns the ventilatory parameters were mostly within the normal range after extubation; therefore, large differences between treatment groups are not to be expected. Furthermore, in this study by modelling an energetic optimal breathing pattern in newborns with RDS we have shown that the sensitivity of tidal breathing parameters is not sufficiently high to detect small changes in lung mechanics.

In contrast to the first RFTs immediately after extubation, at day 28 there were significant differences in lung function between infants with and without BPD. In infants with BPD, respiratory frequency was increased and tidal volume reduced. These changes in the tidal breathing pattern are probably caused by impaired lung mechanics, apparent from the significantly reduced $V_T/maxP_{es}$. However, there is no measurable effect of early Ambroxol treatment on the lung function at day 28.

As shown previously, early Ambroxol therapy reduces the incidence of BPD in VLBW infants with established RDS (9). We speculated that this decrease may have been caused by a reduction of essential BPD risk factors i.e. a reduction in supplemental oxygen, a shorter duration of respiratory support and a lower incidence of infections. The effect of this treatment is explained by an improved supply of surfactant for alveoli and also by the anti-oxidative and anti-inflammatory activities of Ambroxol (5,6).

However, as in early endotracheal surfactant substitution, in the present study, early Ambroxol treatment showed no influence on lung function in infants with BPD. Contrary to expectation, the introduction of surfactant in the treatment of immature surfactant-deficient lungs in VLBW infants failed to sufficiently decrease the incidence of BPD (20–22). Rojas *et al*. (23) have shown that some of the observed infants, with initially mild respiratory disorders, developed a progressively increased respiratory insufficiency with need for mechanical ventilation. This worsening in the clinical course may be caused by a second inflammatory disease which develops beyond the first week of life, independent of the initial postnatal cause of respiratory failure (24,25). These results are in agreement with the present study in which no significant difference in the lung function was found immediately after extubation in infants with and without BPD, whereas we found significant differences in all investigated respiratory parameters at day 28. We speculate that in infants who developed BPD the progressive activity of late inflammatory processes damages the lung tissue, leading to impaired lung function at day 28. This may explain the main results of this study, that early Ambroxol therapy reduces the incidence of BPD but has no influence on inflammatory processes beyond the first week of life which can lead to BPD.

In the present study, Ambroxol was given over a period of 5 days and a long-term effect cannot be expected. Possibly, the deterioration of lung function in infants who subsequently developed BPD could be reduced by prolonged Ambroxol treatment over a longer period. However, no reports exist about such treatment in preterm infants. The study has shown furthermore that the RFT is a valuable tool for assessment of lung development provided that the equipment is well adapted for measurements in preterm infants.

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