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Dose Response to Aerosolized Perflubron in a Neonatal Swine Model of Lung Injury

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ABSTRACT

Aerosolized perfluorocarbon (PFC) improves gas exchange, lung mechanics, and pulmonary artery pressure. The objective of this intervention was to study the dose-response effect to aerosolized perfluorooctylbromide (PFOB; perflubron, LiquiVent, Alliance Pharmaceutical Corp.) in surfactant-depleted piglets. After induction of lung injury by saline lavage, 25 newborn piglets were randomly assigned to receive 0, 1.25, 2.5, 5.0, or 7.5 mL/kg aerosolized PFOB per hour. A 2-h therapy period was followed by a 3-h observation period. In all animals, respiratory support was performed with intermittent mandatory ventilation. After aerosol treatment and 3 h of observation, arterial oxygen pressure was similarly improved in the 2.5-, 5.0-, and 7.5-mL · kg^{-1} · h^{-1} aerosol-PFOB groups and higher compared with the 1.25-mL · kg^{-1} · h^{-1} aerosol-PFOB ($P < 0.01$) and the control groups ($P < 0.001$). Compared with the control group, arterial carbon dioxide pressure was significantly reduced with 2.5-, 5.0-, and 7.5-mL · kg^{-1} · h^{-1} aerosol-PFOB ($P < 0.001$). Treatment with 1.25 mL · kg^{-1} · h^{-1} aerosol-PFOB did not significantly affect arterial carbon dioxide pressure. The 20% terminal dynamic compliance/dynamic compliance was significantly improved in the groups that received 2.5, 5.0, and 7.5 mL · kg^{-1} · h^{-1} aerosol-PFOB compared with control animals. Mean pulmonary artery pressure was lower after therapy with 5.0 and 7.5 mL · kg^{-1} · h^{-1} aerosol-PFOB ($P < 0.01$) than in the control group. IL-1β gene expression in lung tissue was significantly reduced with PFOB 1.25 mL · kg^{-1} · h^{-1}. In summary, aerosolized PFOB improved terminal dynamic compliance, pulmonary gas exchange, and pulmonary artery pressure in a dose-dependent manner. In terms of oxygenation and lung mechanics, the optimum dose was between 2.5 and 5 mL · kg^{-1} · h^{-1}. **(*Pediatr Res* 56: 191–197, 2004)**

Abbreviations

F_{IO_2}, fraction of inspired oxygen
FRC, functional residual capacity
IMV, intermittent mandatory ventilation
LV, low-volume
MPAP, mean pulmonary artery pressure
$Paco_2$, arterial carbon dioxide pressure
Pao_2, arterial oxygen pressure
PEEP, positive end expiratory pressure
PFC, perfluorocarbon
PFOB, perfluorooctylbromide
PIP, peak inspiratory pressure
PLV, partial liquid ventilation

Partial liquid ventilation (PLV) with perfluorocarbons (PFCs) has been developed to ameliorate gas exchange in severe respiratory distress syndrome (1). Respiratory distress syndrome highly contributes to deaths that occur during intensive care treatment. In preterm infants, surfactant deficiency, often in combination with infections, leads to respiratory distress syndrome with the need for intensive respiratory support. Mechanical ventilation itself can induce pulmonary injury *via* shear stress forces, initiating an inflammatory reaction (2–4). This inflammatory process may finally induce irreversible lung damage. In preterm infants, this sequence of events results in chronic lung disease known as bronchopulmonary dysplasia (5). This disease cannot yet be completely prevented by lung protective ventilation strategies such as high-frequency oscillatory ventilation (6). PLV was intended not only to improve oxygenation but also to reduce ventilator-induced lung injury. High gas transport capacity, low surface tension, and chemical inertness make PFCs suitable for respiratory support. PFCs have been used for partial liquid ventilation in animal models as well as in preterm infants (7,8), neonates (9), and adults (10–12). In PLV, lungs are filled with PFC to functional residual capacity and difficulties may occur during the filling and weaning period (13). The question of how to monitor the actual PFC filling volume in the lung and how to regulate PFC substitution for evaporative loss is not yet definitively solved (14–19). Weaning from PLV results in a deterioration in gas

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exchange (20). In contrast, aerosolization, using small volumes of FC77, was shown to improve gas exchange and lung mechanics persistently (20) and to reduce ventilator-associated lung injury at least as potently as PLV (21). Vaporization of perfluorohexane has been shown to improve gas exchange in oleic acid–induced lung injury in sheep (22). This technique, using two anesthetic vaporizers calibrated for PFC, requires PFCs with high vapor pressure, such as perfluorohexane (vapor pressure 273 mm Hg at 30°C). So far, no data exist on the efficacy of aerosolized perfluorooctylbromide (PFOB), which has been purified to medical grade and which is the only PFC that has been available for large, controlled, clinical trials on partial liquid ventilation (13) (perflubron, LiquiVent, Alliance Pharmaceutical Corp.). Differences in vapor pressure or kinematic viscosity between PFOB and FC77 [which has been applied in previous aerosol experiments (20,21)] may influence the aerosol properties. The efficacy of aerosolized PFC delivery might be modified by the evaporation rate of PFC from the lung, by the distribution of PFC within the airways, or by differences in the size of droplets reaching bronchioli or alveolar space.

Aerosolized FC77 showed a significant therapeutic effect at a dose of 10 mL · kg^{-1} · h^{-1}. Because of differences in molecular structure and lower vapor pressure, the optimal dose for aerosolized PFOB could differ from that of FC77. Differences in vapor pressure implies different evaporation rates. The optimal dose of PFOB needed for effective aerosolization therapy has not yet been investigated. Therefore, we designed a dose-response study for aerosolized PFOB in surfactant-depleted piglets.

METHODS

Subjects. Twenty-five piglets with a body weight of 3.3–4.2 kg were included in the study. The animal experiment was approved by the Animal Care Committee of the university and the government of Mittelfranken, Germany, and performed according to the guidelines of the National Institutes of Health. Data of all piglets were available for clinical evaluation.

Animal preparation, lung injury, and therapy groups. Operative preparation, anesthesia, and paralysis of animals [ketamine (15 mg · kg^{-1} · h^{-1}), fentanyl (0.01 mg · kg^{-1} · h^{-1}), midazolam (1.5 mg · kg^{-1} · h^{-1}), and vecuronium (0.2 mg · kg^{-1} · h^{-1})] were performed as described previously (20,21). Briefly, after tracheotomy, a sheath (4.5 F, check-flo performer introducer set, Cook, Mönchengladbach, Germany) was inserted into the right jugular vein and a thermodilution catheter (4 F; Arrow, Erding, Germany) was inserted into the pulmonary artery. An arterial catheter (20 G; Arrow) was inserted into the left femoral artery. A sensor for online blood gas monitoring (Paratrend 7; Agilent, Böblingen, Germany) was introduced in the arterial catheter. Arterial blood gas analysis was performed every 15 min during treatment and every 30 min thereafter (ABL 330; Radiometer, Copenhagen, Denmark). Intermittent mandatory ventilation (IMV) was performed with a time-cycled, pressure-controlled neonatal respirator (Infant Star 950; Mallinckrodt, Hennef, Germany). During operative preparation, a peak inspiratory pressure (PIP) of 20 cm H_2O, a positive end expiratory pressure (PEEP) of 4 cm H_2O, a fraction of inspired oxygen (F_{IO_2}) of 1.0, and a frequency of 40 breaths/min was used. Before lavage, frequency was augmented to 50 breaths/min. Respiratory gas was warmed to 39°C and humidified (MR 700; AGM Fisher & Paykel, Welzheim, Germany). Dynamic compliance was recorded with a hot-wire anemometer (MIM GmbH, Krugzell, Germany), computed with the neonatal respiration monitoring Florian NRM-200 (MIM). In addition, C20/c (compliance during the last 20% of inspiration/total compliance), a marker of pulmonary overdistension, was computed (23) (neonatal respiration monitoring Florian NRM-200, MIM). In pulmonary overdistension, C20/c is low. Cardiac index was measured by thermodilution method before lavage, before treatment, and at the end of the observation period.

Lung injury was induced by repeated bronchoalveolar lavage (24) using 0.9% NaCl at 39°C, 30 mL/kg per side. During lavage, PIP and PEEP were increased to 32 and 8 cm H_2O, respectively. Lung injury was considered successful when the arterial oxygen pressure (Pa_{O_2}) remained below 80 mm Hg for a period of 60 min. The animals were randomized to five different groups of five animals each, receiving different doses of PFOB (Alliance Pharmaceutical Corp., San Diego, CA, U.S.A.) as aerosol *via* an inhalation catheter (AerProbe; Trudell Medical Inc., London, Canada). The tip of the catheter was placed at the distal end of the endotracheal tube; 100% oxygen flow was used to achieve a driving pressure of 45 PSI.

PFOB aerosol was applied at 0, 1.25, 2.5, 5, and 7.5 mL · kg^{-1} · h^{-1}. Aerosolization of PFC at the distal end of the endotracheal tube leads to an ~100% efficiency of PFC delivery. In all groups, respiratory support was performed with identical respiratory settings (PEEP, 8 cm H_2O; PIP, 32 cm H_2O; F_{IO_2}, 1.0, 50 breaths/min). After 2 h, PFOB application was stopped and IMV was continued for another 3 h. The control group received IMV for 5 h.

Cytokine gene expression in lung tissue. RNA extraction, reverse transcription, and TaqMan PCR were performed as described previously (21,25,26). Gene expression was related to β-actin as housekeeping gene.

Data analysis. Data analysis was performed with Microsoft Office and Graph Pad PRISM. Values are expressed as mean ± SEM. After testing for Gaussian distribution, two-way ANOVA was used for comparison of the groups. In case of significance, Bonferroni *post hoc* test was applied, respectively. For cytokine gene expression, Kruskal-Wallis test was used for comparison between the groups. In case of significance, Dunn's *post hoc* test was applied, respectively. $P < 0.05$ was considered significant.

RESULTS

Aerosol therapy with PFOB was well tolerated. There were no deaths or other unexpected events in any of the groups. Mean weight of the animals was not different between the groups (mean ± SD): control, 3.59 ± 0.3 kg; PFOB 1.25 mL · kg^{-1} · h^{-1}, 3.68 ± 0.3 kg; PFOB 2.5 mL · kg^{-1} · h^{-1}, 3.74 ± 0.2 kg; PFOB 5.0 mL · kg^{-1} · h^{-1}, 3.69 ± 0.1 kg; PFOB 7.5

mL · kg^{-1} · h^{-1}, 3.69 ± 0.3 kg. Mean age of the animals was 11 ± 0.9 d; there was no difference in age between the groups.

Oxygenation. Pao_2 improved significantly in all PFOB-treated groups. Significantly higher values for Pao_2 compared with the control group were noticed in all PFOB-treated groups. Differences started to be significant 210 min after the start of 1.25 mL · kg^{-1} · h^{-1} PFOB aerosol ($P < 0.05$), 30 min after the start of 2.5 mL · kg^{-1} · h^{-1} PFOB aerosol ($P < 0.01$), 60 min after the start of 5 mL · kg^{-1} · h^{-1} PFOB aerosol ($P < 0.05$), and 45 min after the start of 7.5 mL · kg^{-1} · h^{-1} PFOB aerosol ($P < 0.01$). In all PFOB-treated groups, Pao_2 was significantly higher than in the control group at the end of the observation period ($P < 0.001$). Comparing the PFOB-treated groups, Pao_2 was significantly higher with 2.5, 5, and 7.5 mL · kg^{-1} · h^{-1} PFOB than with 1.25 mL · kg^{-1} · h^{-1} (Fig. 1). The cumulative PFOB dose at the point where Pao_2 became significantly different from control was 1.25 mL/kg with PFOB 2.5 mL · kg^{-1} · h^{-1}, 5 mL/kg with PFOB 5 mL · kg^{-1} · h^{-1}, and 5.6 mL/kg with PFOB 7.5 mL · kg^{-1} · h^{-1}. With 1.25 mL · kg^{-1} · h^{-1} PFOB, values became significant only after the end of treatment (PFOB evaporation not taken into account).

CO_2 removal. No significant changes in arterial carbon dioxide tension were seen with 1.25 mL · kg^{-1} · h^{-1} PFOB. With all other doses, $Paco_2$ fell significantly compared with control animals. The difference started to be significant for 2.5 mL · kg^{-1} · h^{-1} PFOB at 75 min after therapy start ($P < 0.05$), for 5 mL · kg^{-1} · h^{-1} PFOB at 90 ($P < 0.05$), and for 7.5 mL · kg^{-1} · h^{-1} PFOB at 105 min ($P < 0.01$) after the start of therapy. At the end of the observation period (starting at 270 min), there were no differences between the groups after spontaneous improvement in $Paco_2$ in the control group. The cumulative PFOB dose at the point where $Paco_2$ became significantly different from control was 3.1 mL/kg with PFOB 2.5 mL · kg^{-1} · h^{-1}, 7.5 mL/kg with PFOB 5 mL · kg^{-1} · h^{-1}, and 13.1 mL/kg with PFOB 7.5 mL · kg^{-1} · h^{-1} (PFOB evaporation not taken into account). Details are presented in Fig. 2.

Pulmonary artery pressure. Mean pulmonary artery pressure (MPAP) was significantly different from control animals using 5 and 7.5 mL · kg^{-1} · h^{-1} PFOB. With 5 mL · kg^{-1} · h^{-1} PFOB, MPAP was significantly lower than in the control group at 90 min of therapy ($P < 0.01$) and with 7.5 mL · kg^{-1} · h^{-1} PFOB at 45 min ($P < 0.05$) of therapy. MPAP was not significantly different from the control group in the 1.25 and 2.5 mL · kg^{-1} · h^{-1} PFOB groups. Further details are shown in Fig. 3

Arterial pressure. Mean arterial pressure was not affected in any of the treatment groups compared with the control group.

Dynamic compliance. Although dynamic compliance tended to be higher with 2.5, 5, and 7.5 mL · kg^{-1} · h^{-1}, significant differences compared with the control group were not found (Fig. 4). C20/c was significantly different from control animals in all PFOB-treated groups. Differences to the control group were not significant before 120 min (7.5 mL · kg^{-1} · h^{-1} PFOB, $P < 0.05$) and 150 min (2.5 and 5 mL · kg^{-1} · h^{-1} PFOB, $P < 0.05$ and 0.01, respectively) after therapy start. Details are shown in Fig. 5.

Cardiac index. Cardiac index (L · min^{-1} · m^{-2}) before lavage/before treatment/at the end of the observation period (mean ± SEM) was 0.65 ± 0.11/0.73 ± 0.06/0.81 ± 0.08 in the control group, 0.78 ± 0.07/0.85 ± 0.06/0.83 ± 0.09 with PFOB 1.25 mL · kg^{-1} · h^{-1}, 0.73 ± 0.08/0.69 ± 0.06/0.53 ± 0.06 with PFOB 2.5 mL · kg^{-1} · h^{-1}, 0.67 ± 0.03/0.68 ± 0.07/0.47 ± 0.03 with PFOB 5 mL · kg^{-1} · h^{-1}, and 0.80 ± 0.01/0.72 ± 0.09/0.55 ± 0.04 with PFOB 7.5 mL · kg^{-1} · h^{-1} (NS).

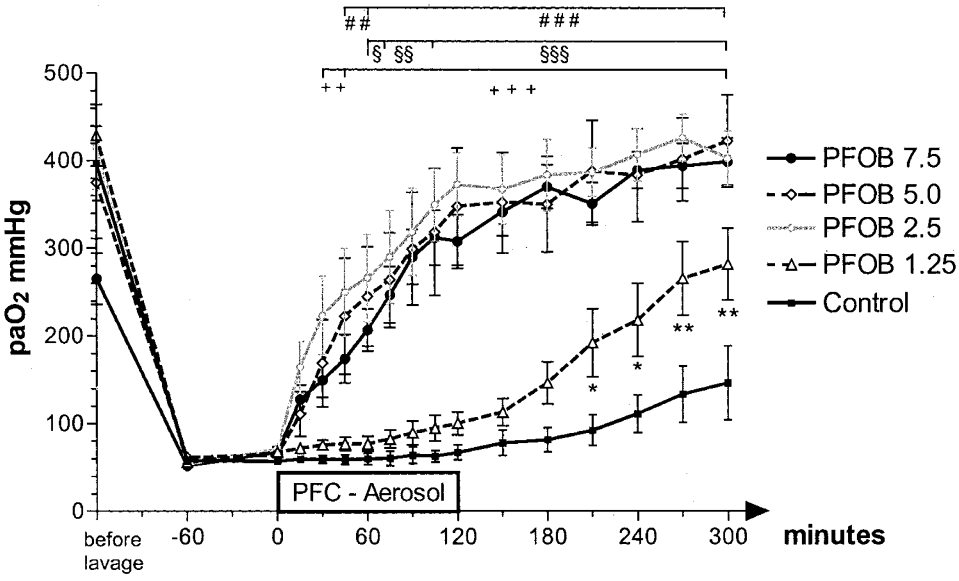

Figure 1. Arterial oxygen tension (Pao_2) in surfactant-depleted piglets before and after induction of lung injury by bronchoalveolar lavage; before treatment with aerosolized PFOB; during a 2-h treatment period with aerosolized PFOB at a dose of 1.25 ($n = 5$), 2.5 ($n = 5$), 5 ($n = 5$), or 7.5 ($n = 5$) mL · kg^{-1} · h^{-1}; and during a 3-h observation period or during 5 h of IMV (Control; $n = 5$). Control *vs* PFOB 1.25 mL · kg^{-1} · h^{-1}: *$P < 0.05$, **$P < 0.01$; control *vs* PFOB 2.5 mL · kg^{-1} · h^{-1}: ++$P < 0.01$, +++$P < 0.001$; control *vs* PFOB 5.0 mL · kg^{-1} · h^{-1}: §$P < 0.05$, §§$P < 0.01$, §§§$P < 0.001$; control *vs* PFOB 7.5 mL · kg^{-1} · h^{-1}: ##$P < 0.01$, ###$P < 0.001$.

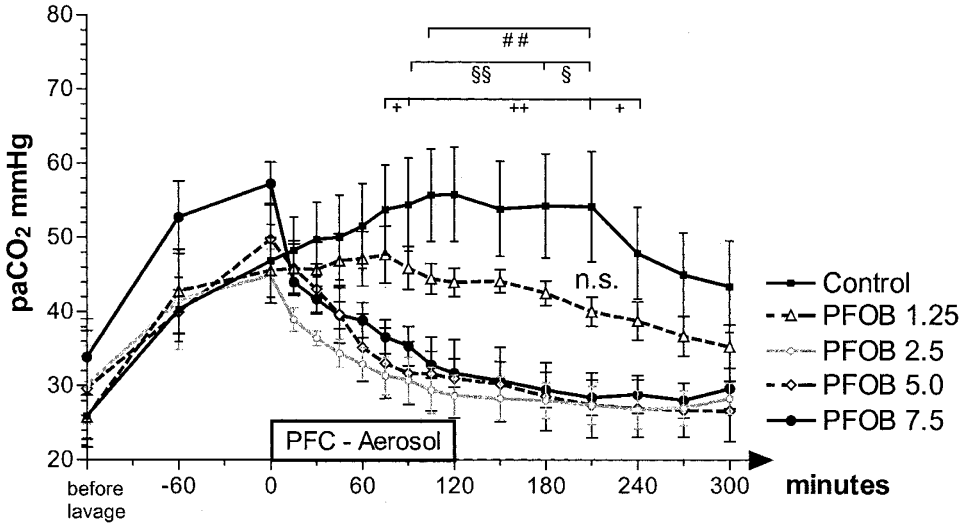

Figure 2. Arterial carbon dioxide tension ($Paco_2$) in surfactant-depleted piglets before and after induction of lung injury by bronchoalveolar lavage; before treatment with aerosolized PFOB; during a 2-h treatment period with aerosolized PFOB at a dose of 1.25 ($n = 5$), 2.5 ($n = 5$), 5 ($n = 5$), or 7.5 ($n = 5$) mL · kg^{-1} · h^{-1}; and during a 3-h observation period or during 5 h of IMV (Control; $n = 5$). Control *vs* PFOB 1.25 mL · kg^{-1} · h^{-1}: NS; control *vs* PFOB 2.5 mL · kg^{-1} · h^{-1}: $+P < 0.05$, $++P < 0.01$; control *vs* PFOB 5.0 mL · kg^{-1} · h^{-1}: §$P < 0.05$, §§$P < 0.01$; control *vs* PFOB 7.5 mL · kg^{-1} · h^{-1}: ##$P < 0.01$.

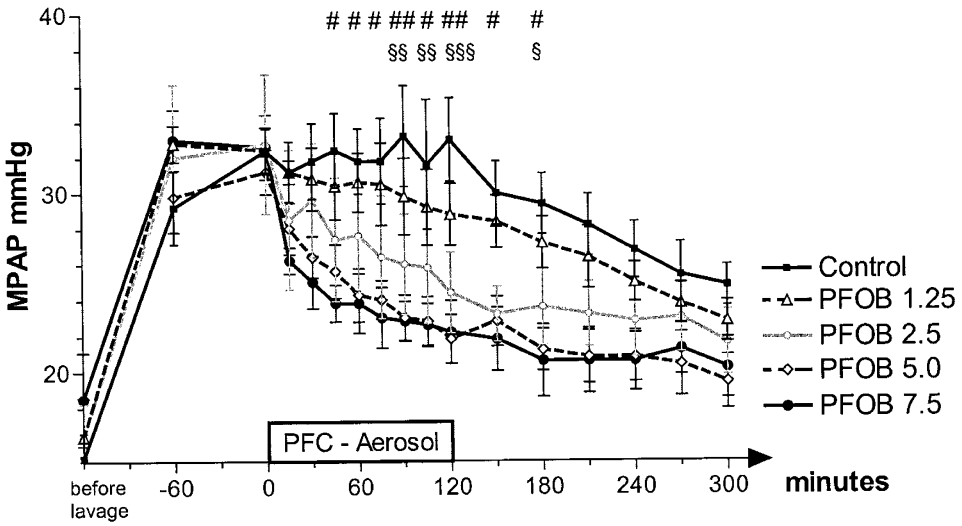

Figure 3. MPAP in surfactant-depleted piglets before and after induction of lung injury by bronchoalveolar lavage; before treatment with aerosolized PFOB; during a 2-h treatment period with aerosolized PFOB at a dose of 1.25 ($n = 5$), 2.5 ($n = 5$), 5 ($n = 5$), or 7.5 ($n = 5$) mL · kg^{-1} · h^{-1}; and during a 3-h observation period or during 5 h of IMV (Control; $n = 5$). Control *vs* PFOB 1.25 mL · kg^{-1} · h^{-1}: NS; control *vs* PFOB 5.0 mL · kg^{-1} · h^{-1}: § $P < 0.05$, §§ $P < 0.01$, §§§ $P < 0.001$; control *vs* PFOB 7.5 mL · kg^{-1} · h^{-1}: #$P < 0.05$, ##$P < 0.01$.

Cytokine gene expression. IL-1β/β-actin mRNA expression (relative units, mean ± SEM) was 2.54 ± 0.43 in the control, 0.79 ± 0.13 in the PFOB 1.25 ($P < 0.001$ *versus* control, $P < 0.05$ *versus* 2.5 and 7.5 mL · kg^{-1} · h^{-1}), 1.16 ± 0.15 in the PFOB 2.5, 1.59 ± 0.28 in the PFOB 5, and 1.36 ± 0.21 in the PFOB 7.5 mL · kg^{-1} · h^{-1} groups.

DISCUSSION

In the present study, the effect of aerosolized PFOB on gas exchange and lung mechanics was dose dependent. The optimal dose for PFOB aerosol was within the dose range investigated (1.25–7.5 mL · kg^{-1} · h^{-1}). Concerning oxygenation and C20/c, 2.5 mL · kg^{-1} · h^{-1} PFOB aerosol is sufficient for therapy of acute respiratory distress syndrome in this experimental model. However, higher doses (5 mL · kg^{-1} · h^{-1}) are required for reduction of pulmonary arterial pressure. Possible explanation for this observation remains speculative. PFOB aerosol at a dose of 2.5 mL · kg^{-1} · h^{-1} might dilate blood vessels in well-ventilated alveoli, whereas PFOB aerosol at a dose of 5 mL · kg^{-1} · h^{-1} might also dilate blood vessels in less ventilated alveoli. In contrast to inhaled vasodilating agents such as nitric oxide (27) or iloprost (28), reaching predominantly ventilated lung areas, PFOB aerosol might additionally recruit collapsed alveoli for ventilation. This would lead to consecutive vasodilation of corresponding pulmonary arterial vessels. However, recruitment of alveoli has not been measured directly. The difference in dose response of oxygenation and pulmonary arterial pressure might show that the reduction

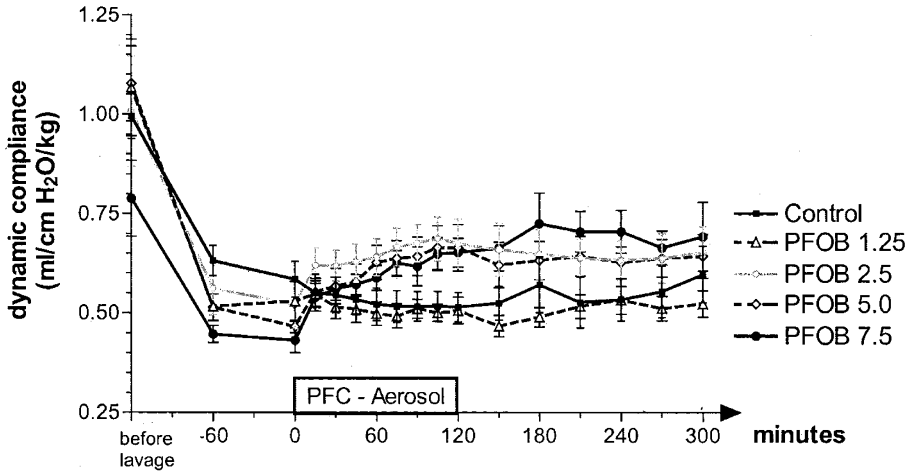

Figure 4. Dynamic compliance (mL · cm H_2O^{-1} · kg^{-1}) in surfactant-depleted piglets before and after induction of lung injury by bronchoalveolar lavage; before treatment with aerosolized PFOB; during a 2-h treatment period with aerosolized PFOB at a dose of 1.25 ($n = 5$), 2.5 ($n = 5$), 5 ($n = 5$), or 7.5 ($n = 5$) mL · kg^{-1} · h^{-1}; and during a 3-h observation period or during 5 h of IMV (Control; $n = 5$).

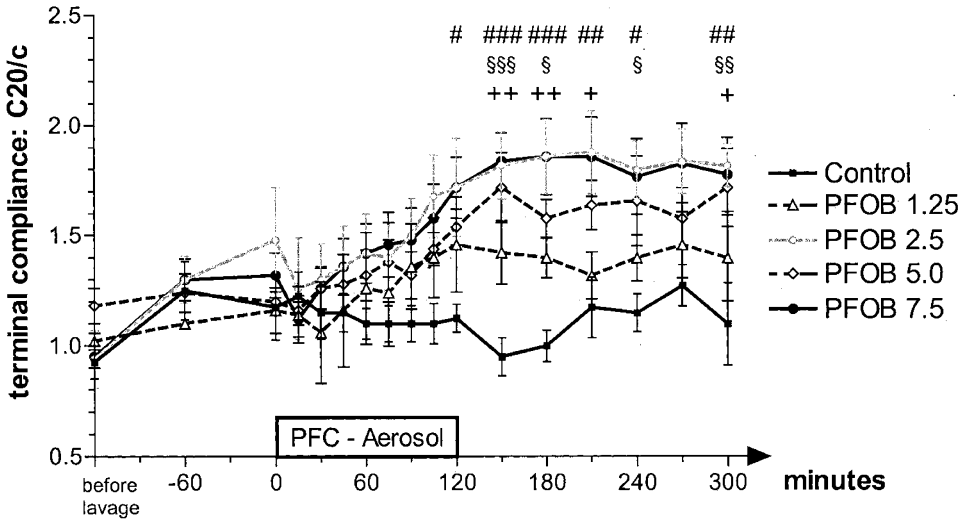

Figure 5. C20/c in surfactant-depleted piglets before and after induction of lung injury by bronchoalveolar lavage; before treatment with aerosolized PFOB; during a 2-h treatment period with aerosolized PFOB at a dose of 1.25 ($n = 5$), 2.5 ($n = 5$), 5 ($n = 5$), or 7.5 ($n = 5$) mL · kg^{-1} · h^{-1}; and during a 3-h observation period or during 5 h of IMV (Control; $n = 5$). Control *vs* PFOB 1.25 mL · kg^{-1} · h^{-1}: NS; control *vs* PFOB 2.5 mL · kg^{-1} · h^{-1}: $+P < 0.05$, $++P < 0.01$; control *vs* PFOB 5.0 mL · kg^{-1} · h^{-1}: §$P < 0.05$, §§$P < 0.01$, §§§$P < 0.001$; control *vs* PFOB 7.5 mL · kg^{-1} · h^{-1}: #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$.

of pulmonary arterial pressure by aerosolized PFOB is not mediated exclusively by oxygen-induced pulmonary vasodilation. Other vasodilative mechanisms of PFOB, such as reduction of surface tension with consecutively improved compliance, could have been effective in this model. A direct vasodilating mechanism of PFOB has not yet been described and should not be concluded from the data. The data clearly demonstrate that a dose of 7.5 mL · kg^{-1} · h^{-1} is not of further benefit. However, no adverse effects could be seen with this dose during this short-term experiment. In the PFOB 7.5 mL · kg^{-1} · h^{-1} group, the difference in Pa_{O_2} ($P < 0.001$: 7.5 mL · kg^{-1} · h^{-1} *versus* the other groups) and in dynamic compliance ($P < 0.01$: 7.5 *versus* 1.25 and 5 mL · kg^{-1} · h^{-1}) before lavage does not necessarily indicate a different degree of health of the animals but might only indicate a different degree of lung recruitment, because in the healthy state, no recruitment maneuver was performed in any of the groups. Otherwise, a different degree of health might have influenced the results. A PFOB dose between 2.5 and 5 mL · kg^{-1} · h^{-1} seems to be sufficient in terms of improvement in oxygenation and lung mechanics. PFC vapor can influence tidal volume measurements with pneumotachographs. Because measurement of compliance is proportional to the measured tidal volume, dynamic compliance is influenced by PFC vapor passing through the pneumotachograph. A falsely high tidal volume as a result of PFC vapor in the pneumotachograph might suggest an improvement of compliance. In a comparable experimental setting, the error in expiratory tidal volume measurements as a result of PFC present in the expired air was ~8% (preliminary data). By calculating terminal dynamic compliance (dividing terminal 20% of dynamic compliance/total dynamic compliance), this error will be minimized. The improvement in C20/c

suggests a reduction of pulmonary overdistension in the treatment groups that received PFOB at a dose of 2.5, 5, or 7.5 mL $\cdot$ kg^{-1} $\cdot$ h^{-1}.

IL-1β mRNA expression was reduced by treatment with aerosolized PFOB. However, a significant reduction compared with control animals was seen only with PFOB 1.25 mL $\cdot$ kg^{-1} $\cdot$ h^{-1}. This may be explained by the short treatment and observation period of only 5 h.

It should be noted that this study on dose-response relationship has been performed in an animal model of neonatal piglets with a body weight of ~3–4 kg. Dose-response might be markedly different in an adolescent or adult situation. If continuously more PFC was applied than the amount evaporating from the lungs, then PFC might accumulate to liquid levels, leading to a changing intrapulmonary distribution of aerosolized and liquid PFC over time. Therefore, overdosing of aerosolized PFC may lead to many of the problems associated with partial liquid ventilation, *e.g.* in terms of deterioration as a result of inadequate filling volume and in terms of weaning. This may be of considerable importance if therapy with aerosolized PFC is performed for a treatment period longer than 2 h. In contrast to a rapid deterioration of oxygenation after the end of PLV, a persistent improvement of gas exchange and lung mechanics was seen in animals that were treated with aerosolized PFC (20). Therefore, aerosolization of PFC could become a therapeutic option in a clinical setting. The best equilibrium of PFOB aerosol administration and evaporation in a long-term treatment period cannot be established with the data of the present study. Few studies have investigated the effect of liquid low-dose PFOB therapy (29). Doses as low as 3 mL/kg of PFOB were shown to be effective when applied in surfactant-depleted piglets on high-frequency oscillatory ventilation (30). However, a filling volume of 30 mL/kg of PFOB was shown to be more effective than 15 mL/kg in preterm lambs (31). After intratracheal administration of 5–25 mL/kg of liquid PFOB in 5-mL/kg doses, Pa_{O_2} was significantly different from the control group only after administration of 15–25 mL/kg in adult pigs (32). In neonatal piglets, PLV with a filling volume of full residual capacity was more effective than a volume of half residual capacity in a bypass model (33). Compared with these data, the optimum dose of ~2.5–5 mL/kg of PFOB for aerosolization is considerably low and highly effective. With regard to previously published data obtained with aerosolized FC77 (20), no significant difference was found for gas exchange between animals that were treated with FC77 (10 mL $\cdot$ kg^{-1} $\cdot$ h^{-1}) in the previous study and PFOB (2.5, 5, and 7.5 mL $\cdot$ kg^{-1} $\cdot$ h^{-1}) in the present study.

In summary, a dose-response relationship was seen with aerosolized PFOB for gas exchange and lung mechanics. An aerosol dose between 2.5 and 5 mL/kg could be considered as appropriate in neonatal surfactant-depleted piglets. A sustained benefit of treatment with aerosolized PFOB was seen for hours after the end of PFOB application.

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