Intensive Care Med (2001) 27: 889–897
DOI 10.1007/s001340100921

EXPERIMENTAL

M. Ragaller
J. Bleyl
U. Tschö
T. Winkler
M. Regner
S. Rasche
T. Koch
M. Albrecht

Effects of inhalation of perfluorocarbon aerosol on oxygenation and pulmonary function compared to PGI_2 inhalation in a sheep model of oleic acid-induced lung injury

Received: 5 July 2000
Final revision received: 19 January 2001
Accepted: 23 January 2001
Published online: 12 April 2001

Financial support: no outside funding was provided. Funding came only from within the department.

M. Ragaller (✉)
Department of Anaesthesiology and Intensive Care Medicine,
University Hospital Carl Gustav Carus, Medical Faculty, Technical University of Dresden, Fetscherstrasse 74,
01307 Dresden, Germany
E-mail: ragaller@rcs.urz.tu-dresden.de
Phone: +49-351-4582785
Fax: +49-351-4584336

J. Bleyl · U. Tschö · S. Rasche
Department of Anaesthesiology and Intensive Care Medicine,
Technical University of Dresden, Dresden, Germany

T. Winkler · M. Regner
Department of Anaesthesiology and Intensive Care Medicine,
Technical University of Dresden, Dresden, Germany

T. Koch
Department of Anaesthesiology and Intensive Care Medicine, Technical University of Dresden, Dresden, Germany

Michael Albrecht
Department of Anaesthesiology and Intensive Care Medicine,
Technical University of Dresden, Dresden, Germany

Abstract *Objective*: To evaluate the effects of PFC aerosol compared to PGI_2 aerosol and NaCl aerosol on gas exchange and lung mechanics in oleic acid-induced acute lung injury.
Design: A prospective, controlled, randomised, in vivo animal laboratory study.
Setting: Research laboratory at an university hospital.
Subjects: Twenty one ($n = 21$) adult sheep of either gender weighing 26.8 ± 6.4 kg.
Interventions: The animals were randomised to three groups: PFC aerosol (perfluorooctane), PFC group; prostacyclin aerosol (Flolan), PGI_2 group; and NaCl aerosol (0.9 % sodium chloride solution), control group. After induction of anaesthesia and placement of vascular catheters, lung injury was induced with 0.12 $ml \cdot kg^{-1}$ oleic acid. Aerosols were continuously administered for 2 h using a jet nebuliser. Gas exchange, pulmonary mechanic, and haemodynamic parameters were obtained at regular intervals.
Measurements and main results: PFC aerosol increased oxygenation (PaO_2) 15 min after the initiation of treatment up to 120 min ($P < 0.05$). Transpulmonary shunt improved in the PFC group ($P < 0.05$) while it did not change in the two other groups. PFC aerosol reduced maximum airway pressure (P_{max}) (median) significantly from (median) 38 mbar to 32 mbar ($P < 0.05$). Static compliance improved significantly in the PFC group ($P < 0.05$).
Conclusion: The inhalation of a PFC aerosol led to a significant improvement in pulmonary mechanics and gas exchange, which was not observed in the other two groups. These data suggest that a small dose of perfluorocarbon will have beneficial effects on gas exchange and respiratory mechanics. Therefore, the non-invasive aerosol application technique seems to be a reasonable alternative to administer perfluorocarbons in severe lung injury.

Key words Acute lung injury · Perfluorocarbons · Aerosol · Jet nebuliser · Prostacyclin · Oleic acid · Low surface tension · Surfactant

Introduction

The pulmonary route as a method of drug delivery is readily accessible during mechanical ventilation. In acute lung injury or acute respiratory distress syndrome (ARDS) [1, 2] substances such as nitric oxide (NO), surfactant, prostacyclin (PGI_2), zaprinast, and perfluorocarbons (PFCs) are administered via this route for therapeutic purposes [3, 4, 5, 6, 7, 8, 9, 10]. This mode of application might be of advantage because of the direct contact with the target organ. Furthermore, as in case of prostacyclin or zaprinast, systemic side effects of the intravenous route are avoided. Perfluorocarbons directly applied into the alveolar space are very promising substances in the treatment of experimental lung injury. Due to their specific characteristics such as low surface tension, and high oxygen and carbon dioxide solubility, they are attractive substances for intra-alveolar application. PFCs are usually applied into the alveolar space of injured lungs as liquids in form of total liquid ventilation (TLV) or partial liquid ventilation (PLV). In experimental settings both techniques were associated with a significant improvement in gas exchange and mechanical lung function [11, 12, 13, 14, 15]. Clinical trials with PLV have shown an improvement in oxygenation as well as some unexpected adverse side effects linked to the mode of PFC application as a liquid. Transient hypoxic events during dosing episodes, as well as pneumothoraces, and PFC leakage into the pleural cavity occurred due to bulk movement of the liquid PFC [16, 17]. Therefore, alternative PFC application techniques should be developed to take advantage of the positive effects of PFC and avoid the risks involved in liquid application. Recently, our group was able to demonstrate an improvement of oxygenation and lung mechanics due to vaporised PFC in experimental ARDS [18]. Devices for aerosol application are widely used in mechanically ventilated patients. Since aerosol therapy with PGI_2 was successful in improving oxygenation and pulmonary hypertension in patients with ARDS [5, 19, 20], this technique may also be practicable for the application of perfluorocarbons. Therefore, we hypothesised that the application of a PFC aerosol may be an alternative technique for alveolar PFC application. The aim of the present study was to investigate the effects of PFC aerosol inhalation on oxygenation, lung mechanics, and pulmonary perfusion in an oleic acid-induced model of ARDS and to compare it to an established mode of aerosol treatment in ARDS.

Materials and methods

The study was performed after approval by the ethics committee of the University Hospital, and with permission from the local government (Regierungspräsidium Dresden, AZ 75–9168.11–1.19/96) in accordance with the "Deutsche Tierschutzgesetz" and the Helsinki convention for the use and care of animals.

Animal preparation

Adult sheep of either gender ($n = 21$) weighing 26.8 ± 6.4 kg were premedicated with xylazine hydrochloride (0.4 mg) (Rompun 2%, Bayer, Leverkusen, Germany). Anaesthesia was induced with midazolam (0.2 mg · kg^{-1}) (Dormicum, Hoffmann-LaRoche, Grenzach, Germany), ketamine (1–2 mg · kg^{-1}), and pancuronium (0.1 mg · kg^{-1}) (CuraMED, Karlsruhe, Germany). Anaesthesia was maintained with a continuous infusion of ketamine (8 mg · kg^{-1} · h^{-1}), midazolam (0.7 mg · kg^{-1} · h^{-1}), and pancuronium (0.05 mg · kg^{-1} · h^{-1}). After intubation (endotracheal tube 8.0 mm; Mallinckrodt, Athlone, Ireland), mechanical ventilation was performed using a Servo 900C (Siemens-Elema, Solna, Sweden) in a volume-controlled mode at a rate of 20 min^{-1}, tidal volume of 10 ml · kg^{-1}, PEEP of 5 mbar, FiO_2 of 1.0, and an inspiratory/expiratory ratio of 1:1. To achieve normocapnia, respiratory frequency was adjusted according to blood-gas analysis. During inhalation of the different aerosols, tidal volume and total inspiratory flow rate remained unchanged.

Fluid losses were replaced by intravenous infusion of crystalloid solution (E153, AWD, Dresden, Germany) and colloid solution (HES 6%, Fresenius, Bad Homburg, Germany) at a rate of 5–10 ml · kg^{-1} · h^{-1}. ECG was derived by needle electrodes. The left common carotid artery was surgically exposed and cannulated with an 18 G arterial line (Vygon, Ecouen, France) to obtain arterial blood pressure as well as arterial blood samples. The arterial cannula as well as a pulmonary artery catheter (8 Fr, Abbott, Ill., USA) were connected to pressure transducers (Ohmeda, Erlangen, Germany). The p.a. catheter was inserted into the left jugular vein and advanced into the pulmonary artery to obtain pulmonary artery pressure (PAP), pulmonary capillary wedge pressure (PCWP), central venous pressure (CVP), core body temperature, and mixed venous blood samples. Both pressure transducers were referenced to atmospheric pressure at the level of the right atrium. Cardiac output (CO) was measured by the conventional thermodilution technique using bolus injection of 10 ml cold saline solution (5–10 °C) and calculated as the mean of three measurements arbitrarily performed during different phases of the respirator cycle. Extravascular lung water (EVLW) and intrathoracic blood volume (ITBV) were measured by a thermal dye dilution technique using the COLD-System (COLD Z-02; Pulsion Medizintechnik, Munich, Germany). Therefore, an artery catheter equipped with infrared fibreoptics and a thermistor (4F-FT-PULSIOKATH, Pulsion Medizintechnik, Munich, Germany) was inserted in the left carotid artery. For measurements, 10 ml glucose 5% containing 1.0 mg · ml^{-1} indocyanine green dye at 0–4 °C was injected into the right atrium per bolus injection [21]. A urinary catheter was inserted to determine the fluid balance. In addition, an orogastric tube was inserted to alleviate gastric distension. The animals remained in the prone position throughout the experiment.

Preparation of drugs

Perfluorooctane

The perfluorocarbon used in this study – perfluorooctane (C_8F_{18}) (ABCR, Karlsruhe, Germany) – is a purified substance (90%). This substance is characterised by the following chemical data: molecular weight 438 g, density 1.74 g · ml^{-1}, vapour pressure of

60.9 mmHg at (37 °C), boiling point 100–105 °C, oxygen solubility 52.1 $mlO_2 \cdot 100ml^{-1}$, viscosity 0.85 $cs \cdot °C^{-1}$, surface tension 13.9 $dyn \cdot cm^{-1}$. Perfluorooctane is a clear, colourless, and chemically inert liquid which is not miscible with aqueous solutions.

Prostacyclin (PGI_2)

The crystalline epoprostenol (0.5 mg) ([5Z,9α,11α,13E,15S]-6,9-epoxy11,15-dihydroxyprosta-5,13-dien-1-oic-acid) (Flolan, Glaxo Wellcome Pharma, Vienna, Austria) was dissolved in 50 ml aqueous glycine buffer solution of pH 10.5, which led to a concentration of 10,000 $ng \cdot ml^{-1}$. Immediately before use, this solution was diluted to the appropriate concentration (1,500 $ng \cdot ml^{-1}$) with normal saline. This solution was used directly for aerosolisation and resulted in dose of 50 $ng \cdot kg^{-1} \cdot min^{-1}$.

Isotonic sodium chloride solution (0.9 % NaCl)

In the control group sterile saline solution (0.9 % NaCl, Fresenius, Bad Homburg, Germany) was nebulised.

Administration of the aerosols

Aerosolization was achieved with the help of a synchronised electromagnetic gas valve (Servo Nebulizer 945, Siemens Elema, Solna, Sweden) connected to the Servo 900C ventilator. The nebuliser gas flow (100 % O_2) synchronised by an inspiratory signal from the ventilator was directed to a standard nebuliser chamber, which was positioned in the inspiratory limb just proximal the Y-piece (Cirrus, Intersurgical, St Augustin, Germany). To avoid disconnecting the ventilator circuit (loss of PEEP) the chamber was modified to allow permanent refilling during aerosolisation. Using this particular nebulising chamber with flow rates of 8 $l \cdot min^{-1}$, aerosol particles with a diameter of 0.5–10 μm with a mean particle size of 3.5 μm MMD (mass median diameter) (aqueous solutions) were created (manufacturers information). The flow rate through the chamber in these experiments was 14 $l \cdot min^{-1}$ which further reduces the particle size of the aerosol. The size of the particles was not measured in this study; however, as shown by Zwissler et al., this respirator and nebuliser setting creates an aerosol with a mean particle size of 3.1 μm MMD [29]. Due to the different chemical properties, perfluorocarbon aerosols created by the described setting may differ in particle size if compared to aqueous solutions. Therefore, we can only assume that there are no major differences in the particle sizes between aqueous and PFC solutions. This issue has to be addressed in further studies.

Experimental protocol

After surgical preparation and a stabilisation period of 30 min, $PaCO_2$ was adjusted to between 5.0–5.8 kPa. Thereafter, baseline measurements were taken. Lung injury was induced by injecting of 0.12 ml oleic acid $(C_{18}H_{34}O_2) \cdot kg^{-1}$ body weight emulsified in 15–20 ml of previously extracted blood over 30 min into the right atrium. Severe lung injury was considered established when the PaO_2/FiO_2 ratio was < 200 and the PCWP was < 19 mmHg [2]. Previous studies had shown that this protocol results in a severe lung injury after 30–90 min [22].

After fulfilling the criteria of severe lung injury the three different therapeutic regimens were implemented in a randomised order:

1. PFC group ($n = 7$): inhalation of perfluorooctane (C_8F_{18}); 1.3 $ml \cdot min^{-1}$ (2.8 $ml \cdot kg^{-1} \cdot h^{-1}$).
2. Prostacyclin group (PGI_2 group) ($n = 7$): inhalation of a median dose of 50 $ng \cdot kg^{-1} \cdot min^{-1}$ epoprostenol.
3. Control group ($n = 7$): inhalation of warmed sterile NaCl solution 0.9 % 1.3 $ml \cdot min^{-1}$.

The animals in the three different groups were treated for 120 min. All animals were killed after the end of the experiment.

Measurements and data acquisition

Haemodynamic data were collected every 10 s using a Merlin monitoring system (Hewlett Packard, Böblingen, Germany). A complete set of measurements was obtained at baseline, at the time of established lung injury, 15 min and 30 min after therapy onset, and at 30 min intervals thereafter until the end of the experiment (120 min). Pulmonary vascular resistance (PVR) was calculated as (mean PAP-PCWP/CO · 79,9 = PVR $dyn \cdot s \cdot cm^{-5}$).

Arterial and mixed venous blood samples were taken with heparin-coated syringes and determined by a blood gas analyser (AVL-995-S, Graz, Austria). Transpulmonary shunt (Qsp/Qt) was calculated according to the standard equation: $Qsp/Qt = (CcO_2 - CaO_2)/(CcO_2 - CvO_2)$. Respiratory parameters such as tidal volume (V_T), peak inspiratory pressure (P_{max}), inspiratory plateau- and mean pressures, and PEEP were measured by an integrated strain gauge transducer within the Servo 900C ventilator. Tidal volumes were measured in the expiratory limb of the ventilator by a strain gauge flow transducer. Static lung compliance was calculated using $C = V_T/(Ppl\text{-}PEEP) \cdot kg^{-1}$ body weight, where V_T is the tidal volume, Ppl is the end-inspiratory plateau pressure, and PEEP is the positive end-expiratory pressure.

Statistics

Data are presented as median, Q1/Q3 quartiles, and minimum and maximum in the figures and as median and minimum and maximum in the table. Baseline values were analysed separately with Student's *t*-test. Statistical analysis was performed using an ANOVA for repeated measurements corrected with Bonferroni's procedure (SPSS 10.07, statistical software). With this program, effects of groups, effects of time, and interactions between time and groups were analysed. The premises for the analyses were tested. A P value of < 0.05 was taken as a significant level [23].

Results

At baseline the animals were haemodynamically stable and comparable in their pulmonary situation. There was merely a significant difference in mPAP between the PFC group and the two other groups (Table 1). Injection of oleic acid induced acute lung injury after approximately 75 min in all animals. After induction of lung injury there were no significant differences between the three groups in the observed parameters (Table 1, Figs. 1, 2, 3, 4, 5).

Table 1 Haemodynamic and respiratory data of the experiments at different measurement points. Measurements at *BL* = baseline, *I* = injury, and 15, 30, 60, 90, and 120 min after onset of treatment. Data shown as median and minimum and maximum

	Group	Baseline	Injury	15 min	30 min	60 min	90 min	120 min
HF(min^{-1})	NaCl	104(85/120)	105(50/146)	124(106/150)	132(110/146)	133.5(106/139)	126(108/149)	127(97/212)
	PFC	96(77/128)	115(83/145)	111(94/150)	120(82/145)	118(86/147)	116(75/150)	118(92/155)
	PGI_2	115(87/129)	104(91/180)	116(93/150)	116(91/149)	124(95/160)	122(108/152)	123(94/148)
MAP(mmHg)	NaCl	106.5(95/132)	96(49/124)	94.5(54/128)	92.5(57/135)	90(78/130)	93.5(68/143)	96(93/137)
	PFC	102(94/107)	92(72/143)	98(63/122)	99(79/137)	100(83/127)	101(84/130)	97(45/136)
	PGI_2	106(89/135)	92(73/119)	98(64/121)	92(69/109)	80(68/102)	85(67/107)	69(43/88)
mPAP (mmHg)	NaCl	20.0(10.0/27.0)[a]	33.5(27.0/53.0)	30.0(28.0/38.0)	34.0(31.0/36.0)	34.5(33.0/39.0)	34.0(32.0/45.0)	32.0(31.0/45.0)
	PFC	9.0(8.0/23.0)	29.0(17.0/41.0)	24.0(14.0/43.0)	24.0(13.0/41.0)	25.0(11.0/45.0)	28.0(12.0/44.0)	25.0(12.0/44.0)
	PGI_2	21.0(15.0/25.0)[a]	40.0(24.0/49.0)	33.0(22.0/49.0)	35.0(22.0/54.0)	33.0(23.0/53.0)	42.0(21.0/52.0)	39.0(24.0/50.0)
CVP (mmHg)	NaCl	7.0(1.0/18.0)	6.5(3.0/14.0)	10.0(1.0/16.0)	9.5(4.0/16.0)	8.0(4.0/10.0)	10.0(4.0/12.0)	11.5(4.0/21.0)
	PFC	7.0(5.0/11.0)	7.0(3.0/17.0)	5.0(1.0/16.0)	4.0(2.0/16.0)	5.0(1.0/17.0)	5.0(1.0/17.0)	7.0(1.0/15.0)
	PGI_2	7.0(5.0/13.0)	10.0(4.0/15.0)	10.0(4.0/17.0)	8.0(5.0/16.0)	10.0(5.0/17.0)	14.0(5.0/18.0)	15.0(6.0/22.0)
PCWP (mmHg)	NaCl	12.0(10.0/18.0)	14.5(10.0/18.0)	13.0(10.0/16.0)	13.5(12.0/19.0)	13.5(11.0/16.0)	13.0(9.0/19.0)	13.5(9.0/20.0)
	PFC	8.0(5.0/13.0)	9.0(8.0/16.0)	8.0(2.0/17.0)	10.0(2.0/16.0)	11.0(5.0/17.0)	10.0(1.0/18.0)	11.0(1.0/16.0)
	PGI_2	14.0(10.0/17.0)	14.0(10.0/17.0)	14.0(8.0/18.0)	14.0(10.0/18.0)	13.0(9.0/19.0)	16.0(11.0/18.0)	16.0(10.0/19.0)
ITBV ($ml \cdot m^{-2}$)	NaCl	675(444/776)	468(345/810)	389(141/732)	356(211/465)	458(390/549)	584(424/598)	483(212/558)
	PFC	532(315/964)	421(322/690)	537(337/600)	556(369/722)	463(269/742)	659(356/695)	695(377/799)
	PGI_2	1070(483/1167)	820(364/988)	591(387/776)	638(366/969)	576(376/775)	539(349/728)	617(393/840)
CO ($l \cdot min^{-1}$)	NaCl	2.9(1.8/3.7)	2.2(1.8/3.8)	2.0(1.2/4.0)	2.3(1.0/5.0)	2.5(1.7/3.4)	2.6(1.7/4.4)	3.2(2.1/4.5)
	PFC	2.6(2.0/17.0)	2.5(1.7/15.0)	2.4(1.6/4.1)	2.7(1.3/5.6)	2.8(1.7/5.5)	2.7(2.0/4.9)	3.0(2.0/3.6)
	PGI_2	2.5(1.9/6.4)	2.4(1.3/4.3)	2.1(1.6/6.6)	2.2(1.7/7.3)	2.4(1.7/6.6)	2.8(2.1/5.5)	3.1(1.8/5.4)
PVR ($dyn \cdot s \cdot cm^{-5}$)	NaCl	230(196/317)	705(360/1271)	826(378/876)	744(316/1306)	766(467/970)	672(422/1168)	607(389/714)
	PFC	97(27/303)	415(96/1182)	442(322/1290)	413(269/1248)	404(146/1348)	495(291/975)	416(288/1130)
	PGI_2	170(73/335)	793(293/1967)	733(181/1568)	788(165/1498)	702(133/1319)	802(205/1364)	548(268/1481)
$PaCO_2$ (kPa)	NaCl	6.4(4.9/8.0)	7.3(4.9/10.9)	8.0(5.7/12.1)	9.4(7.2/12.3)	9.7(7.1/15.6)	10.0(7.2/16.8)	9.9(6.8/16.9)
	PFC	5.8(5.3/6.3)	6.4(5.3/11.7)	6.0(4.9/8.9)	6.1(5.0/9.5)	7.1(5.3/11.7)	7.6(5.2/12.1)	7.2(5.5/11.3)
	PGI_2	5.0(4.2/5.9)	7.1(5.2/11.6)	9.8(6.9/11.6)	9.1(8.0/11.5)	9.8(8.2/12.3)	11.7(8.6/15.3)	11.8(10.7/17.0)
EVLWI ($ml \cdot kg^{-1}$)	NaCl	14.6(6.8/23.0)	13.9(11.0/24.7)	19.0(12.3/51.9)	29.3(15.4/56.0)	22.7(16.8/37.2)	19.3(15.5/31.2)	20.3(19.3/30.7)
	PFC	12.6(8.1/14.7)	22.5(16.4/30.4)	21.8(19.3/24.3)	21.7(21.0/22.0)	20.3(19.4/24.6)	20.0(17.0/26.4)	19.5(17.1/28.6)
	PGI_2	6.3(2.5/12.6)	27.5(15.8/27.8)	26.0(14.2/37.7)	16.8(14.6/36.8)	29.3(15.5/43.0)	32.9(18.4/47.3)	36.3(20.9/51.7)

Haemodynamics

There were no significant changes throughout the experiment in heart frequency (HF), mean arterial pressure (MAP), central venous pressure (CVP), pulmonary capillary wedge pressure (PCWP), cardiac output (CO), and intrathoracic blood volume (ITBV) (Table 1.). After induction of lung injury, pulmonary arterial pressure (mPAP), pulmonary vascular resistance (PVR), and extravascular lung water index (EVLWI) increased significantly compared to baseline in all groups similarly and remained elevated throughout the whole observation period (Table 1).

Gas exchange

The administration of PFC aerosol led to a significant increase in PaO_2 from 15 min onwards when compared to the two other groups ($P < 0.05$) (Fig. 1). Maximum PaO_2 (median 27.14 kPa) was reached 90 min after lung injury. The PGI_2 group and the saline group did not change in PaO_2 throughout the therapy phase. To give more detailed information, the PaO_2 of the PFC group is shown in a scatterplot in (Fig. 2). Although the response to treatment presents considerable dispersion there was an improvement in PaO_2 in all animals, with the exception of one animal (animal 3) which had a lower PaO_2 already at baseline (Fig. 2). $PaCO_2$ did not change during the aerosolisation and showed no significant difference between the three groups (Table 1). Inhalation of PFC aerosol reduced transpulmonary shunt over time which reached statistical significance at 90 min ($P < 0.05$) (Fig. 3).

Pulmonary mechanics

The administration of PFC aerosol was associated with a significant improvement of pulmonary mechanics. Peak inspiratory pressure (P_{max}) decreased significantly in the PFC group with respect to injury, 30 min after start of inhalation from (median) 38 mbar to 32 mbar and remained unchanged at this level throughout the

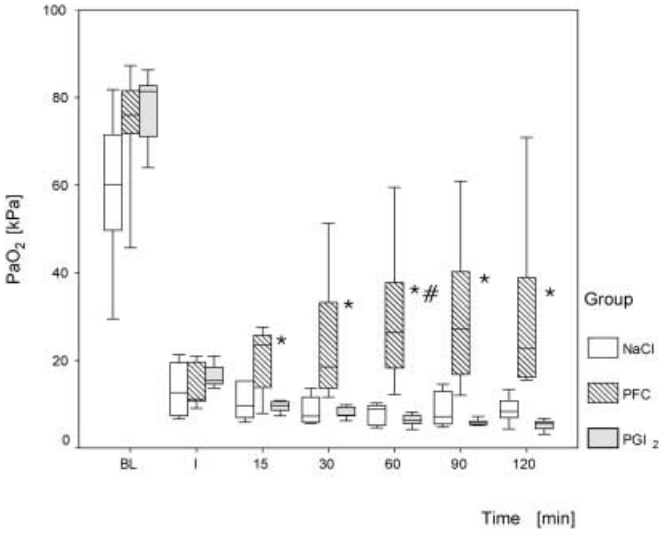

Fig. 1 Arterial oxygen partial pressure PaO_2 (kPa) (y-axis) over time (min) (x-axis). Measurements at *BL* = baseline, *I* = injury, and 15, 30, 60, 90, and 120 min after onset of treatment. Values shown in boxplots (median, Q1/Q3 quartiles, minimum/maximum). NaCl group = *open box*; PFC group = *hatched box*; PGI_2 group = *grey box*. The increase of PaO_2 in the PFC group over time is statistically significant compared to injury ($P < 0.05$) and compared to both other groups. * PFC group significantly different compared to PGI_2 group ($P < 0.05$); # PFC group significantly different compared to NaCl group ($P < 0.05$) at this measurement point

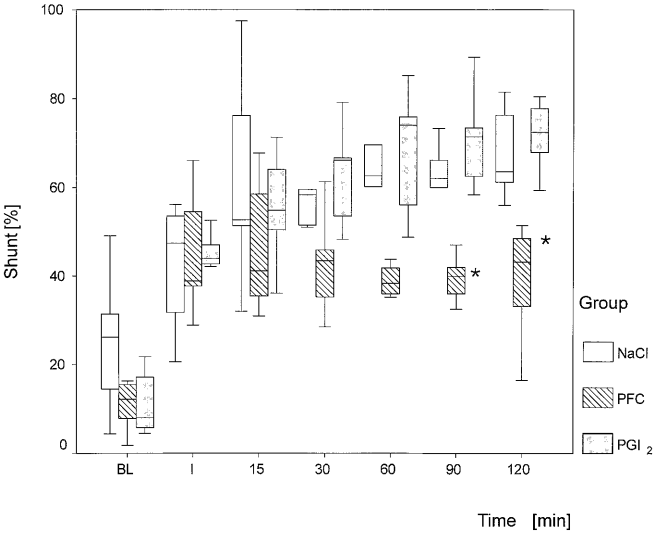

Fig. 3 Transpulmonary shunt (%) (y-axis) over time (min) (x-axis). Measurements at *BL* = baseline, *I* = injury, and 15, 30, 60, 90, and 120 min after onset of treatment. Values shown in boxplots (median, Q1/Q3 quartiles, minimum/maximum). NaCl group = *open box*; PFC group = *hatched box*; PGI_2 group = *grey box*. The difference in transpulmonary shunt reaches statistical significance after 60 min after start of therapy between the PFC group and the PGI_2 group ($P < 0.05$). * PFC group significantly different compared to PGI_2 group ($P < 0.05$); # PFC group significantly different compared to NaCl group ($P < 0.05$) at this measurement point

whole therapy period. In the two other groups there was no change during aerosol inhalation. The course of Pmax of the PFC group over time was significantly different to the two other groups ($P < 0.05$) (Fig. 4). The inhalation of perfluorooctane-aerosol improved static compliance significantly from (median) 0.30 ml · $mbar^{-1} \cdot kg^{-1}$ after injury to 0.37 ml · $mbar^{-1} \cdot kg^{-1}$ after 15 min ($P < 0.05$) (Fig. 5). Improvement of lung compliance in the PFC group was significantly different throughout the whole treatment period as compared to the two other groups. Extravascular lung water index, observed in five animals in each group, consider-

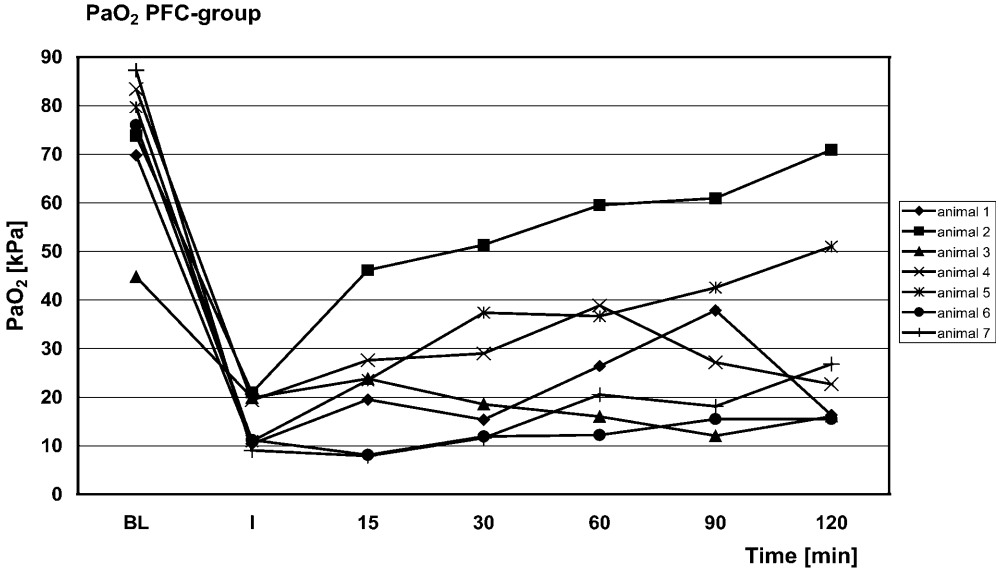

Fig. 2 A scatterplot of PaO_2 of each individual animal of the PFC group over time. Measurements at *BL* = baseline, *I* = injury, and 15, 30, 60, 90, and 120 min after onset of treatment

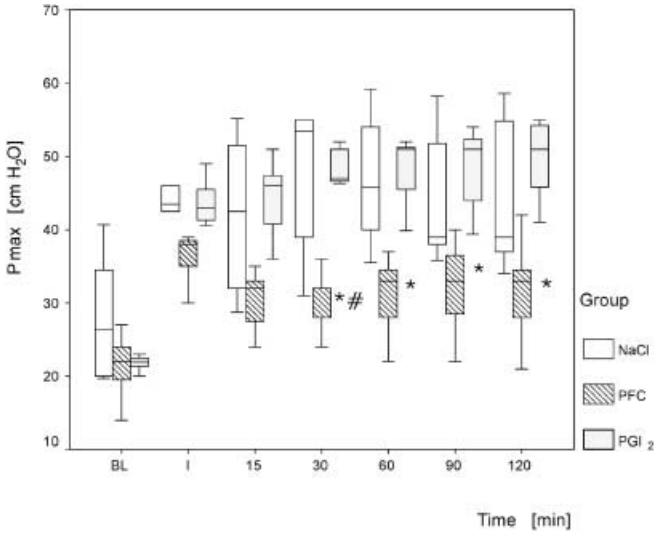

Fig. 4 The peak inspiratory pressure P_{max} (kPa) (y-axis) over time (min) (x-axis). Measurements at *BL* = baseline, *I* = injury, and 15, 30, 60, 90, and 120 min after onset of treatment. Values shown in boxplots (median, Q1/Q3 quartiles, minimum/maximum). NaCl group = *open box*; PFC group = *hatched box*; PGI_2 group = *grey box*. The reduction of P_{max} over time in the PFC group is statistically significant compared to injury ($P < 0.05$) and compared to both other groups ($P < 0.05$). * PFC group significantly different compared to PGI_2 group ($P < 0.05$); # PFC group significantly different compared to NaCl group ($P < 0.05$) at this measurement point

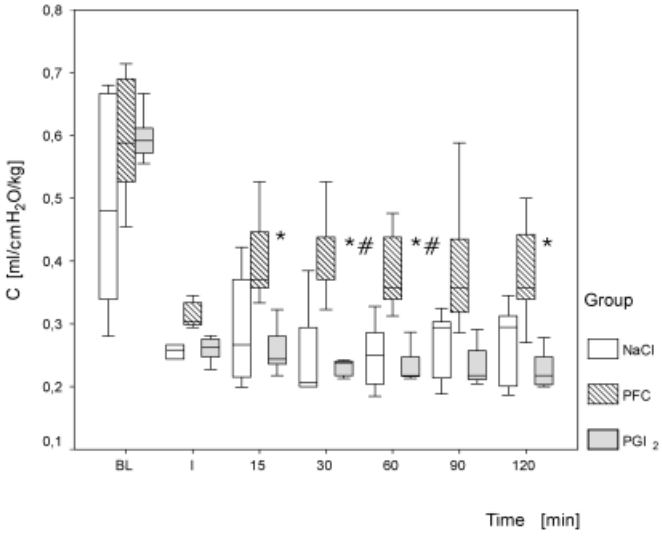

Fig. 5 Static compliance C ($ml \cdot mbar^{-1} \cdot kg^{-1}$) (y-axis) over time (min) (x-axis). Measurements at *BL* = baseline, *I* = injury, and 15, 30, 60, 90, and 120 min after onset of treatment. Values shown in boxplots (median, Q1/Q3 quartiles, minimum/maximum). NaCl group = *open box*; PFC group = *hatched box*; PGI_2 group = *grey box*. The increase of compliance over time in the PFC group is statistically significant compared to injury ($P < 0.05$) and compared to both other groups ($P < 0.05$). * PFC group significantly different compared to PGI_2 group ($P < 0.05$); # PFC group significantly different compared to NaCl group ($P < 0.05$) at this measurement point

ably increased after oleic acid injection without any further change in all groups (Table 1).

Discussion

Perfluorocarbons are well known to improve gas exchange, shunt, and lung compliance in studies of total or partial liquid ventilation or vaporisation [11, 12, 13, 14, 15, 18]. In contrast, their effects as therapeutic aerosols are poorly investigated. The current paper reports on the effects of inhalation of perfluorooctane aerosol compared to prostacyclin aerosol and saline aerosol in an ovine model of oleic acid-induced ARDS. The oleic acid model was selected because it is known for its reliability in causing severe lung injury and has been repeatedly used to study the consequences of lung injury as well as in evaluating treatment strategies [22]. The perfluorocarbon mostly used for partial liquid ventilation is perfluorooctylbromide ($C_8F_{17}Br$), which is not freely available for experimental or clinical purpose. Therefore, we used a PFC (perfluorooctane) with similar physico-chemical properties. Furthermore, perfluorooctane had been used for PLV successfully [24, 25].

At baseline all animals were comparable. The higher mPAP in the two control groups at baseline might indicate that there was a considerable lung injury already. However, after induction of lung injury there was no difference between the groups. Therefore, this difference in mPAP is probably accidental and has no influence on the results of the aerosol therapy.

The inhalation of perfluorocarbon aerosol has no influence on haemodynamic parameters such as heart rate, mean arterial pressure, mean pulmonary pressure, wedge pressure, cardiac output or intrathoracic blood volume. Looking at the haemodynamic parameters, some results in the PGI_2 group were unexpected. In the current study, inhalation of PGI_2 aerosol had no effect on mean pulmonary arterial pressure or gas exchange as described in previous studies [5, 8, 19, 26, 27]. This result might be explained by the severity of injury and by the extent of pulmonary vasoconstriction induced by mediators like thromboxane (TXA_2). In a canine study, Welte et al. were not able to counteract the effects of a thromboxane analogue by PGI_2 aerosol inhalation up to dosages of 50,000 $ng \cdot kg^{-1} \cdot min^{-1}$ [28]. Second, oleic acid leads to pulmonary arterial hypertension induced by pulmonary microembolism. This pathogenesis might be resistant to PGI_2 treatment, or might need a higher dosage of PGI_2 to reduce pulmonary arterial pressure. This hypothesis is in concordance with data by Zwissler et al. who were not able to improve mPAP in a model of oleic acid-induced lung injury by PGI_2 inhalation [29]. Furthermore, there is no clear effective dosage of PGI_2 described in the literature. The

dosage varies between 1 ng·kg^{-1} · min^{-1} and 10,000 ng · kg^{-1} · min^{-1} in different studies and species [5, 8, 19, 20, 27, 28, 29]. In a study in children with severe ARDS, the same nebulised dose PGI_2 (20 ng · kg^{-1} · min^{-1}) had different effects in the same child if applied at different time points [19]. Furthermore, due to the aerosol technique, there is a difference between the nebulised amount of drug and effective amount in the alveolar space. It is known from different studies that, depending on the technique, approximately 2–22 % of the nebulised amount will effectively reach the alveolar surface [10, 30, 31, 32].

The improvement in gas exchange and pulmonary mechanics were frequently seen in PLV [11, 12, 13]. However, using the technique of liquid ventilation, usually large amounts of perfluorocarbon liquid are administrated into the alveolar space, which may compromise cardiac output or lung mechanics due to the increasing weight of the liquid-filled lung [15, 33, 34]. Administrating drugs by inhalation as an aerosol or as a vapour as demonstrated by our group recently [18], has the advantage of presenting small amounts to the alveolar space of ventilated lung regions without these liquid-bulk effects. The small particles of PFC fluid generated by nebulisation are transported by the inspiratory gas flow to the surface of the alveoli. Due to their specific characteristics (low surface tension, positive spriting coefficient, oxygen transport) the perfluorocarbon may form a new air-liquid interface. However, it is thought that therapeutic aerosols must reach the alveolar space to be effective. Depending on placement of the nebuliser in the respiratory circuit, gas flow through the nebuliser chamber, respiratory frequency, tidal volume, type of the nebuliser, and duration of the aerosol application, the dose that actually reaches the alveoli oscillates between 2 % and 22 % of the amount delivered into the nebuliser chamber [30, 31, 32]. Although we exactly measured the amount of substance that was aerosolised in the chamber, we do not know the amount of PGI_2 or PFC that actually reached the targeted alveolar surface. The loss of substance can be due to trapping on walls of the nebuliser chamber, ventilator, and endotracheal tubing, tracheal surface or simply by exhalation [10, 19, 30, 31, 32]. To overcome some of these drawbacks of aerosol therapy, we used a high flow, continuous-filled, jet nebuliser placed in the inspiratory limb of the ventilator circuit as proposed by O'Doherty [31]. We did not use an ultrasonic nebuliser because of the unknown effects of high energy ultrasonic oscillations on the perfluorocarbon molecule with a possible release of fluoride radicals.

In this current study we demonstrated an improvement in gas exchange and ventilatory mechanics due to inhalation of perfluorooctane compared to the other groups. Oxygenation started to increase in the PFC group after 15 min and reached a maximum after 90 min of PFC inhalation (Fig. 1). The improvement over time suggests a cumulative effect of PFC inhalation. However, as shown in the scatterplot of PaO_2, the effect of PFC aerosol on PaO_2 is notably variable. Whereas six animals improved in PaO_2, animal 3 did not improve over time (Fig. 2). This response might be influenced by an impaired gas exchange already existing at baseline. The variable improvement of PaO_2 might be caused by the dispersion of the severity of lung injury in this model. A second explanation are the different amounts of PFC reaching the alveolar surface in the different animals which led to the different effects of PFC aerosol. After 120 min, a total amount of approximately 150 ml perfluorocarbon was nebulised and therefore a dose of 5.6 ml · kg^{-1} could be calculated as the applied dose. However, a smaller amount of PFC might have reached the alveolar space as discussed already. This increase in oxygenation with a low dose of PFC is in contrast to results from PLV where a dosage of at least 10 ml · kg^{-1} is proposed to improve oxygenation [33, 34]. However, in early studies from Tütüncü and colleagues in rabbits, a dose of 3–6 ml · kg^{-1} led to a marked increase in oxygenation [13]. Recently, Baden and co-workers demonstrated the effectiveness of 3 ml · kg^{-1} of perflubron combined with high-frequency oscillatory ventilation in improving gas exchange [35]. The current study is also in concordance with the results of a study with perfluorocarbon vapour in the same model. Though using a different mode of application (vapour vs aerosol), the effects of both techniques are comparable [18]. Whereas the effects of these two techniques are comparable, the aerosol technique might be easier to establish in ICU than the more complex technique of PFC vaporisation. These data suggest that it is not imperative or necessary to fill up the lung with large amounts of perfluorocarbon liquid up to 20–30 ml · kg^{-1} b.w. (FRC dose) to achieve improvement in gas exchange.

Besides the positive effects on gas exchange, inhalation of PFC aerosol improves peak inspiratory pressure (P_{max}) and static lung compliance (C) significantly. The effects on pulmonary mechanics are known to be dose-independent since small dosages of 3 ml · kg^{-1} b.w. led to a significant reduction of P_{max} as described previously [13, 18, 24, 25, 33, 34]. These effects could not be further enhanced by increasing the dosage in partial liquid ventilation [13]. Furthermore, in experimental studies with higher amounts of liquid applied to the lungs > 10 ml · kg^{-1}, an increase in P_{max} and a decrease in compliance were even observed probably caused by the increasing weight of the liquid-filled lungs [15, 33, 34].

The mechanism of action of perfluorocarbons in the alveolar space is still not fully elucidated and remains speculative. Using the concept of liquid ventilation, several mechanisms like recruitment of atelectatic alveoli,

prevention of end-expiratory alveolar collapse ("liquid PEEP"), redistribution of perfusion, oxygen transport, surfactant-like effects, and anti-inflammatory effects are currently being discussed [33, 36, 37, 38, 39]. Perfluorocarbons applied to the lungs as bulk liquid will preferentially reach dependent lung areas [40], whereas perfluorocarbons administrated as an aerosol will probably reach all ventilated areas. Due to their low surface tension, the perfluorocarbons may spread over the surface and build a thin layer of PFC in the alveoli and reduce surface tension. Considering the hydrophobic nature of perfluorocarbons, we furthermore speculate that perfluorocarbons interact with the hydrophobic parts of the lipid shares such as dipalmitoyl phosphatidylcholine (DPPC) or with hydrophobic surfactant proteins like SP-B or SP-C which are mostly responsible for surface activity [41, 42]. Additionally, due to its lipid solubility, perfluorooctane may interact with the lipid layer of the cell membrane of the alveolar cells. The solubility of PFCs in the lipid layer of the cell membrane was demonstrated in erythrocytes by Obrastzow et al. recently [43]. Another explanation is the stimulation of surfactant production or release of stored surfactant from alveolar type II cells by PFC as postulated by Steinhorn et al. [39]. Therefore, we hypothesise a thin PFC-layer with high oxygen transport capacity and surface activity which may stabilise the alveoli. This finally results in better alveolar ventilation and in improved oxygenation and compliance over a period of time. Furthermore, the stabilisation of alveolar surface with a hydrophobic layer may prevent or reduce alveolar oedema.

Conclusion

In conclusion, the administration of a perfluorooctane aerosol to lungs using a jet nebuliser leads to an improvement in pulmonary mechanics and gas exchange and is therefore superior to PGI_2 in this model of severe ARDS. These data suggest that even a low dose of perfluorocarbons applied in the aerosol form may have beneficial effects on gas exchange and respiratory mechanics. Compared to the invasive technique of total or partial liquid ventilation with high doses of liquid perfluorocarbon, the aerosol technique seems to be a practicable alternative to administer perfluorocarbons in severe lung injury. The presumed mechanism of a specific interaction between the hydrophobic perfluorocarbon and hydrophobic parts of the natural surfactant has to be elucidated in further studies.

Appendix

Transpulmonary shunt fraction (shunt) (Qsp/Qt) was calculated according to the standard equation: $Qsp/Qt = (CcO_2 - CaO_2)/(CcO_2 - CvO_2)$, where Qsp is the physiologic shunt, Qt is the cardiac output, CaO_2 is the oxygen content of the arterial blood, CvO_2 is the oxygen content of mixed venous blood, and CcO_2 is the oxygen content of blood draining from the ideal alveolus ventilated with gas of an $FiO_2 = 1.0$. CcO_2 was calculated as $CcO_2 = Hb \cdot 1.39 + 0.0031 \cdot PaO_2$ where PaO_2 is the alveolar oxygen partial pressure. PaO_2 was calculated as $PaO_2 = FiO_2 \cdot (Pbar\text{-}47) - PaCO_2/0.8$ where Pbar is the barometric pressure at the day of the experiment.

References

1. Ashbaugh DG, Bigelow DB, Petty TL, Levine BE (1967) Acute respiratory distress in adults. Lancet 11: 319–323
2. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, Legall JR, Morris A, Spragg R (1994) The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial co-ordination. Am J Respir Crit Care Med 149: 818–824
3. Roissant R, Gerlach H, Schmidt-Runke H, Pappert D, Lewandowski K, Steudel W, Falke K (1995) Efficacy of inhaled nitric oxide in patients with severe ARDS. Chest 107: 1107–1115
4. Roissant R, Falke KJ, Lopez F, Slama K, Pison U, Zapol WM (1993) Inhaled nitric oxide for the adult respiratory distress syndrome. N Engl J Med 328: 399–405
5. Walmrath D, Schneider T, Pilch J, Schermuly R, Grimminger F, Seeger W (1995) Effects of aerosolized prostacyclin in severe pneumonia: impact of fibrosis. Am J Respir Crit Care Med 151: 742–50
6. Lewis JF, Goffin J, Yue P, McCaig LA, Bjarneson D, Veldhuizen RAW (1996) Evaluation of exogenous surfactant treatment strategies in an adult model of acute lung injury. J Appl Physiol 80: 1156–1164
7. Tashiro K, Yamada K, Li WZ, Matsumoto Y, Koyabyashi T (1996) Aerosolized and instilled surfactant therapies for acute lung injury caused by intratracheal endotoxin in rats. Crit Care Med 24: 488–494
8. Welte M, Zwissler B, Habazettl H, Messmer K (1993) PGI_2 Aerosol versus nitric oxide for selective pulmonary vasodilation in hypoxic vasoconstriction. Eur Surg Res 25: 329–340
9. Zwissler B, Welte M, Messmer K (1995) Inhalation of vasodilatory drugs or gases. Curr Op Anaesthesiol 8: 557–564
10. Ichinose F, Adrie C, Hurford WE, Bloch K, Zapol WM (1998) Selective pulmonary vasodilation induced by aerosolized zaprinast. Anesthesiology 88: 410–6
11. Hirschl RB, Pranikoff T, Wise C, Overbeck MC, Gauger P, Schreiner RJ, Dechert R, Bartlett RH (1996) Initial experience with partial liquid ventilation in adult patients with the acute respiratory distress syndrome. JAMA 275: 383–389
12. Curtis SE, Peek JT, Kelly DR (1993) Partial liquid breathing with perflubron improves arterial oxygenation in acute canine lung injury. J Appl Physiol 75: 2696–2702

13. Tütüncü AS, Faithfull NS, Lachmann B (1993) Intratracheal perfluorocarbon administration combined with mechanical ventilation in experimental respiratory distress syndrome: dose-dependent improvement of gas exchange. Crit Care Med 21: 962–969
14. Fuhrman BP, Paczan PR, DeFrancis M (1991) Perfluorocarbon-associated gas exchange. Crit Care Med 19: 712–722
15. Hirschl RB, Tooley R, Parent A, Johnson K, Bartlett RH (1996) Evaluation of gas exchange, pulmonary compliance, and lung injury during total and partial liquid ventilation in the acute respiratory distress syndrome. Crit Care Med 24: 1001–1008
16. Bartlett RH, Croce M, Hirschl RH, Gore D, Wiedemann H, Davis K, Zwischenberger J (1997) A phase II randomized, controlled trial of partial liquid ventilation (PLV) in adult patients with acute hypoxemic respiratory failure (AHRF). Crit Care Med [Suppl] 25:A35
17. Cox PN, Frndova H, Tan PS, Nakamura T, Miyasaka K, Sakurai Y, Middleton W, Mazer D, Bryan AC (1997) Concealed air leak associated with large tidal volumes in partial liquid ventilation. Am J Respir Crit Care Med 156: 992–997
18. Bleyl JU, Ragaller M, Tschö U, Regner M, Kanzow M, Hübler M, Rasche S, Albrecht DM (1999) Vaporized perfluorocarbon improves oxygenation and pulmonary function in an ovine model of acute respiratory distress syndrome. Anesthesiology 91: 477–485
19. Pappert D, Busch T, Gerlach H, Lewandowski K, Radermacher P, Rossaint R (1995) Aerosolized prostacyclin versus inhaled nitric oxide in children with severe acute respiratory distress syndrome. Anesthesiology 82: 1507–1511
20. Van Heerden PV, Barden A, Michalopoulos N, Bulsara MK, Roberts BL (2000) Dose response to inhaled aerosolized prostacyclin for hypoxemia due to ARDS. Chest 117: 819–827
21. Lichtwark-Aschoff M, Zeravik J, Pfeiffer UJ (1992) Intrathoracic blood volume accurately reflects circulatory volume status in critically ill patients with mechanical ventilation. Intensive Care Med 18: 142–147
22. Schuster DP (1994) ARDS: clinical lessons from the oleic acid model of acute lung injury. Am J Respir Crit Care Med 149: 245–260
23. Winer BJ, Brown DR, Michels KM (1991) Statistical principles in experimental design 3rd edn. McGraw-Hill, New York
24. Kaisers U, Max M, Walter J, Kuhlen R, Pappert D, Falke K, Rossaint R (1997) Partial liquid ventilation with small volumes of FC 3280 increases survival time in experimental ARDS. Eur Respir J 10: 1955–1961
25. Kaisers U, Max M, Schnabel R (1996) Partial liquid ventilation with FC 3280 in experimental respiratory distress syndrome: dose-dependent improvement of gas exchange and lung mechanics. Appl Cardiopulm Pathophysiol 6: 163–170
26. Walmrath D, Schneider T, Schermuly R, Olschewski H, Grimminger F, Seeger W (1996) Direct comparison of inhaled nitric oxide and aerosolized prostacyclin in acute respiratory distress syndrome. Am J Respir Crit Care Med 153: 991–996
27. Zwissler B, Kemming G, Habler O, Kleen M, Merkel M, Haller M, Briegel J, Welte M, Peter K (1996) Inhaled prostacyclin (PGI_2) versus inhaled nitric oxide in adult respiratory distress syndrome. Am J Respir Crit Care Med 154: 1671–1677
28. Welte M, Zwissler B, Habler O, Kleen M, Messmer K (1995) Prostacyclin aerosol and inhaled nitric oxide fail to reverse pulmonary vasoconstriction induced by thromboxane analogue in dogs. Acta Physiol Scand 154: 395–405
29. Zwissler B, Welte M, Habler O, Kleen M, Messmer K (1995) Effects of inhaled prostacyclin as compared with inhaled nitric oxide in a canine model of pulmonary microembolism and oleic acid edema. J Cardiothor Vasc Anesth 9: 634–640
30. O'Riordan TG, Palmer LB, Smaldone GC (1994) Aerosol deposition in mechanically ventilated patients. Am J Respir Crit Care Med 149: 214–219
31. O'Doherty ML, Thomas SLH, Page CJ, Treacher DF, Nunan TO (1992) Delivery of a nebulized aerosol to a lung model during mechanical ventilation effect of ventilator settings and nebulizer type, position, and volume of fill. Am Rev Respir Dis 146: 383–388
32. Thomas SL, O'Doherty ML, Page CJ, Treacher DF, Nunan TO (1993) Delivery of ultrasonic nebulized aerosols to a lung model during mechanical ventilation. Am Rev Respir Dis 148: 872–877
33. Quintel M, Meinhardt J, Waschke KF (1998) Partielle Flüssigkeitsventilation (partial liquid ventilation). Anaesthesist 47: 479–489
34. Overbeck MC, Pranikoff T, Yadao CM, Hirschl RB (1996) Efficacy of perfluorocarbon partial liquid ventilation in a large animal model of acute respiratory failure. Crit Care Med 24: 1208–1214
35. Baden HP, Mellema JD, Bratton SL, O'Rourke PP, Jackson JC (1997) High-frequency oscillatory ventilation with partial liquid ventilation in a model of acute respiratory failure. Crit Care Med 25: 299–302
36. Shaffer TH, Wolfson MR, Greenspan JS (1999) Liquid ventilation: current status. Pediatr Rev 20: 134–142
37. Ragaller M, Bleyl JU, Koch T, Albrecht DM (2000) Perfluorocarbons-therapeutic strategies in ARDS. From isoflurane to perfluorohexane. Anaesthesist 49: 291–301
38. Thomassen MJ, Buhrow LT, Wiedemann HP (1997) Perflubron decreases inflammatory cytokine production by human alveolar macrophages. Crit Care Med 25: 2045–2057
39. Steinhorn DM, Leach CL, Fuhrman BP, Holm BA (1996) Partial liquid ventilation enhances surfactant phospholipid production. Crit Care Med 24: 1252–1256
40. Quintel M, Hirschl RB, Roth H, Loose R, Tillmanns R, van Ackern K (1998) Computer tomographic assessment of perfluorocarbon distribution and gas distribution during partial liquid ventilation for acute respiratory failure. Am J Respir Crit Care Med 158: 249–255
41. Takahashi A, Waring AJ, Amirkhanian J, Fan B, Taeusch CA (1990) Structure-function relationship of bovine pulmonary surfactant proteins: SP-B and SP-C. Biochim Biophys Acta 1044: 43–49
42. Weaver TE (1998) Synthesis, processing and secretion of surfactant proteins B and C. Biochim Biophys Acta 1408: 173–179
43. Obraztsow VV, Neslund GG, Kornbrust ES, Flaim SF, Woods CM (2000) In vitro cellular effects of perfluorochemicals correlate with their lipid solubility. Am J Physiol Lung Cell Mol Physiol 278:L1018–1024