European Journal of Pharmaceutics and Biopharmaceutics 76 (2010) 75–82

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Semifluorinated alkanes – A new class of excipients suitable for pulmonary drug delivery

C. Tsagogiorgas [a,*], J. Krebs [a], M. Pukelsheim [a], G. Beck [a], B. Yard [b], B. Theisinger [c], M. Quintel [d], T. Luecke [a]

[a] Dept. of Anaesthesiology and Intensive Care Medicine, University Medical Center Mannheim, Mannheim, Germany
[b] V. Dept. of Medicine, University Medical Center Mannheim, Mannheim, Germany
[c] Novaliq GmbH, Heidelberg, Germany
[d] Department of Anaesthesiology, Emergency and Intensive Care Medicine, University of Göttingen, Göttingen, Germany

ARTICLE INFO

Article history:
Received 29 December 2009
Accepted in revised form 31 May 2010
Available online 2 June 2010

Keywords:
Semifluorinated alkanes
Pulmonary drug delivery
Inhalation
Aerosolisation
Respiratory system mechanics
Perfluorocarbons

ABSTRACT

Introduction: Semifluorinated alkanes (SFAs) are considered as diblock molecules with fluorocarbon and hydrocarbon segments. Unlike Perfluorocarbons (PFCs), SFAs have the potential to dissolve several lipophilic or water-insoluble substances. This makes them possibly suitable as new excipients for inhalative liquid drug carrier systems.
Purpose: The aim of the study was to compare physico-chemical properties of different SFAs and then to test their respective effects in healthy rabbit lungs after nebulisation.
Methods: Physico-chemical properties of four different SFAs, i.e. Perfluorobutylpentane (F4H5), Perfluorohexylhexane (F6H6), Perfluorohexyloctane (F6H8) and Perfluorohexyldodecane (F6H12) were measured. Based on these results, aerosol characteristics of two potential candidates suitable as excipients for pulmonary drug delivery, i.e. F6H8 and F4H5, were determined by laser light diffraction. Tracheotomised and ventilated New Zealand White rabbits were nebulised with either a high- or a low dose of SFAs ($F6H8_{low/high}$ and $F4H5_{low/high}$) or saline (NaCl). Ventilated healthy animals served as controls (Sham). Arterial blood gases, lung mechanics, heart rate and blood pressure were recorded prior to nebulisation and in 30 min intervals during the 6-h study period.
Results: Out of the four SFAs studied initially, no satisfactory behaviour as a solvent has to be expected because of low lipophilicity for F6H6. Output rate during aerosolisation was very low for F6H12. F6H8 and F4H5 presented comparable aerosolisation characteristics and lipophilicity and were therefore tested in the in vivo model. Aerosol therapy, either SFAs or saline, impaired p_aO_2/F_iO_2 ratio, dynamic lung compliance and respiratory mechanics in all groups, except for $F4H5_{low}$ group which behaved like the control group (Sham). $F4H5_{low}$ had no adverse effects on gas exchange or pulmonary mechanics.
Conclusions: Perfluorobutylpentane (F4H5) in a low-dose application may be suitable as a new inhalable excipient in SFA-based pulmonary drug delivery systems for lipophilic or water-insoluble substances.

1. Introduction

Inhaled aerosols offer significant potential for non-invasive systemic administration of therapeutics but also for direct drug delivery into the diseased lung. Drugs for pulmonary inhalation are typically formulated as solutions, suspensions or dry powders. Aqueous solutions of drugs are common for inhalational therapy [1]. Yet, about 40% of new active substances exhibit low solubility in water, and many fail to become marketed products due to formulation problems related to their high lipophilicity [2,3]. Formulations for drug delivery to the respiratory system include a wide variety of excipients to assist aerosolisation, solubilise the drug, support drug stability, prevent bacterial contamination or act as a solvent [4,5].

Semifluorinated alkanes (SFAs) are colourless non-aqueous liquids consisting of diblock molecules with perfluorocarbon (R_F) and hydrocarbon (R_H) segments with chemical structure $F(CF_2)_m$-$(CH_2)_nH$. The nomenclature of SFAs is simplified in FmHn, where *m* and *n* describe the number of carbon atoms in the fluorocarbon or the hydrocarbon chain, respectively. At first sight, physico-chemical properties of SFAs are similar to those of their distant

Abbreviations: CST, critical solution temperature; F4H5, Perfluorobutylpentane; F6H6, Perfluorohexylhexane; F6H8, Perfluorohexyloctane; F6H12, Perfluorohexyldodecane; GSD, geometric standard deviation; IPPV, intermittent positive pressure ventilation; MMAD, mass median aerodynamic diameter; P_{peak}, peak airway pressure; $PEEP_{tot}$, measured positive end-expiratory pressure; PFC, perfluorocarbon; SFA, semifluorinated alkane; VMD, volume median diameter; Vt, tidal volume.

* Corresponding author. Address: Dept. of Anaesthesiology and Intensive Care Medicine, University Medical Center Mannheim, Medical Faculty Mannheim of the Heidelberg University, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany. Tel.: +49 (0) 621 383 3771; fax: +49 (0) 621 383 2164.
E-mail address: charalambos.tsagogiorgas@umm.de (C. Tsagogiorgas).

0939-6411/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2010.05.011

relative perfluorocarbons (PFCs), e.g. strong intramolecular bonds, weak intermolecular interactions, high gas dissolving capacities and low surface tensions. Similar to PFCs, SFAs are considered to be extremely hydrophobic, also thermal, chemical and biological inert [6,7]. Except for their lower densities, there is one evident difference between SFAs and PFCs; whereas PFCs are extremely hydro- and lipophobic, SFAs are amphiphilic compounds given by the hydrophobic and lipophobic R_F-segment and the hydrophobic and lipophilic R_H-segment. The ratio between R_F- and R_H-segments defines their physico-chemical properties [8,9]. Biocompatibility of SFAs seems to be dependent on their lipophilic behaviour and furthermore on the molecular dimension of the semifluorinated alkanes used [10]. As indicated by Meinert et al. [8], SFAs might be used as solvents or solubilizers for selected drugs in their basic, physiologically effective form instead of their modified, water-soluble hydrochloride. This makes SFAs potentially interesting as new excipients, especially as solvents for lipophilic and water-insoluble drugs and substances for the use in liquid inhalative drug carrier systems. A recent pilot study of our group demonstrated the feasibility of an inhalative non-invasive systemic administration of therapeutics based on a drug-in-SFA solution [11].

As shown for PFCs, differences in vapour pressure and viscosity may also influence generation and behaviour of SFA aerosols. Furthermore, evaporation and intrapulmonary accumulation of PFCs are dependent on vapour pressure [12,13]. Attributes like low vapour pressure, high viscosity and high molecular weight in PFCs lead to low evaporation rates with accumulation in the lung. A reduced pulmonary elimination mimicks liquid ventilation with a relatively low PFC volume inducing negative changes in oxygenation, respiratory system resistance and lung compliance [9,13–15].

Physico-chemical properties of four different SFAs (Perfluorobutylpentane – F4H5, Perfluorohexylhexane – F6H6, Perfluorohexyloctane – F6H8 and Perfluorohexyldodecane – F6H12) were obtained by literature review or in a bench study by own investigations, if not available. Output rates and aerosolisation characteristics were measured. Based on these results, we hypothesised that despite very similar aerosol characteristics compared to F6H8, F4H5 would be eliminated faster because of a high vapour pressure and a low viscosity. This would prevent accumulation in the lungs without leading to adverse effects on lung mechanics and oxygenation. To test this hypothesis, subsequently we performed a confirmatory animal study examining the influence of F4H5 and F6H8 on oxygenation, carbon dioxide removal and pulmonary mechanics in healthy rabbit lungs, in a dose-dependent manner.

2. Methods

This study protocol was approved and monitored by the local animal care committee (Regierungspräsidium Karlsruhe). All procedures were performed according to the Guide for the Care and Use of Laboratory Animals published by the National Academy of Sciences and was approved by the local authorities. Semifluorinated alkanes were obtained in courtesy of Novaliq GmbH, Heidelberg. All chemical reagents were purchased from Sigma Aldrich (Sigma–Aldrich Chemie GmbH, Munich, Germany) unless otherwise indicated.

2.1. Bench study

2.1.1. Measurement of physico-chemical properties

Four different semifluorinated alkanes with different molecular weights were analyzed (Table 1). Determination of density was measured by the oscillating body method (DA-100 M, Mettler-Toledo GmbH, Germany) at 25 °C. Kinematic viscosity was determined by repeated measurements with an Ubbelohde type viscometer (Schott GmbH, Germany) at 25 °C. Dynamic viscosity was calculated as follows:

$$\text{dynamic viscosity} = \text{kinematic viscosity} * \text{density} \quad (1)$$

Vapour pressure was measured with a Minivap VPXpert (Grabner-Instruments, Vienna/Austria) at room temperature. Vapour pressure at 37 °C was calculated according to Clausius–Clapeyron relation and Raoult's law.

A marker for lipophilic properties of SFA and PFC is the critical solution temperature in *n*-bromohexane or in olive oil (CST) – shown in Table 1. Increasing the alkyl part of the molecule makes the compounds more lipophilic; the CST value decreases. Increasing perfluorinated carbon segments in SFA increases molecular weight and vapour pressure as well CST values. Surface and interface tension were adopted according to literature [9,16].

2.1.2. Aerosolisation, output rates and evaluation of droplet sizes

Nebulisation of the test substances was performed with an aerosolisation catheter (AeroProbe®, Trudell Medical International, Ontario, Canada) and an electromechanical catheter control system (LABneb®, Trudell Medical International, Ontario, Canada). The nebulisation catheter consists of a multi-lumen tubular shaft ending in a multi-orifice nozzle tip. Pressurized gas and liquid are transported to the nozzle tip orifices via five gas lumens and one liquid lumen. At the tip, the liquid is pneumatically aerosolised. In preliminary tests, pressures lower than 60 psi (4.14 bar) lead to marginal output rates of all tested substances (data not shown). Therefore, catheters were run with an input pressure of 60 psi (4.14 bar) on both the liquid and air lines with a total continuous flow of 1.4 l/min (manufacturer information). At this pressure, output rates per minute were measured by filling the catheter system with 2 ml followed by time measurement until the end of aeroslisation. Each liquid was tested fivefold.

For evaluation of droplet sizes, the catheter was positioned via a sealed sideport connector at the tip of an endotracheal tube. Volume mass diameter (VMD in μm) and geometric standard deviation (GSD in μm) of aerosolised semifluorinated alkanes and

Table 1
Physico-chemical properties of semifluorinated alkanes used in this study (modification from Kim et al. [16] – new measurements done by Novaliq GmbH, Heidelberg). Semifluorinated alkanes (SFA). F4H5: Perfluorobutylpentane; F6H6: Perfluorohexylhexane; F6H8: Perfluorohexyloctane; F6H12: Perfluorohexyldodecane. Perfluorocarbon PFOB: Perfluorooctylbromide; H_2O: water.

Substance	Molecular weight (g/mol)	Density (g/cm^3) at 25 °C	Boiling point (°C)	Viscosity (mPa) at 25 °C	Vapour pressure (Torr)	CST-*n* bromo hexane (°C)	CST in olive oil (°C)	Surface tension (mN/m)	Interface tension (mN/m)
F4H5	290	1.284	134	1.05	25.1 (37 °C)	≪−30 °C	68	17.43	43.0
F6H6	404	1.386	187	2.38	<1(25 °C)	–	121	20.0	49.6
F6H8	432	1.331	223	3.44	<1(25 °C)	−31	70	19.65	45.3
F6H12	488	1.25	290	6.99	–	–	14	21.1	–
PFOB	499	1.930	143	1.93	10.5 (37 °C)	68	–	18	51.3
H_2O	18	0.970	100	0.89	46.9 (37 °C)	–	–	72	n/a

saline were measured 2.5 cm after catheter exit by directing the aerosol across the beam of a laser diffraction particle sizer (HELOS H2109, Sympatec, Clausthal-Zellerfeld, Germany) using a lens determining droplets between 0.25 and 87.5 μm (R2, Sympatec, Clausthal-Zellerfeld, Germany). The filling volume was 5 ml. Five repetitions of fivefold 20 s continuous measurement followed and were done under moderate elevated ambient temperature of 25 ± 1 °C and a relative humidity of 49 ± 4%. The measurement conditions and other technical information are given in Table 2. Data were evaluated by Sympatec Windox5.3.1.0-Software by using the Mie-Theory with a form factor of 1 (spherical particle). Refraction index was determined with an Abbe-refractometer AR6D (Krüss-Optronic GmbH, Germany) at 20 °C and was set for F4H5 at 1.3204 and for F6H8 at 1.3432. For calculation of deposition efficiency, the mass median aerodynamic diameter (MMAD) was calculated out of the VMD by using the density of 1.284 g/cm^3 for F4H5, 1.331 g/cm^3 for F6H8 and 1.004 g/cm^3 for saline. Since the particle density is directly proportional to the MMAD, the VMD values were multiplied with the density.

2.2. Animal study

2.2.1. Animal preparation and monitoring

Thirty-six New Zealand White rabbits weighing 2.7 ± 0.16 kg were randomized to the various experimental groups by means of concealed envelopes. After randomization, the animals were pre-oxygenated with 100% oxygen by nose cone and anaesthetised with ketamine (25 mg/kg, intramuscularly, Ketanest 10%®, Pfizer, Karlsruhe, Germany) and xylazine (5 mg/kg intramuscularly, Rompun®, BayerVital, Leverkusen, Germany). A 24-gauge peripheral vascular catheter (Becton Dickinson GmbH, Heidelberg, Germany) was placed in the lateral auricular vein. Anaesthetised animals were placed in supine position, the anterior neck was dissected, and a tracheotomy was performed. An uncuffed endotracheal tube (3.5–4.0 inner diameter) with an integrated pressure catheter was placed in the trachea and secured in position. A vascular cannula (BD GmbH, Heidelberg, Germany) was inserted in the common carotid artery, and a 4-Fr 8-cm three-lumen catheter (Arrow Int., Teleflex medical GmbH, Kernen, Germany) was advanced into superior vena cava through the internal jugular vein. Central venous and arterial blood pressures were monitored by attaching the vascular catheters to standard pressure transducers. Heart rate was monitored by electrocardiogram (ECG). Esophageal pressure was monitored by a modified pressure tip catheter (Millar Instruments, Inc., Houston, Texas, USA) placed in the oesophagus under spontaneous breathing. The correct position was verified using the occlusion test [17]. Tidal volume was measured using a pneumotachograph (MIM GmbH, Krugzell, Germany) directly connected to the endotracheal tube. Muscle relaxation was then induced by intravenous administration of pancuronium bromide (0.2 mg/kg) and maintained with 0.1 mg/kg doses as needed to avoid spontaneous breathing efforts. Body temperature was monitored continuously using a rectal probe, and normothermia was maintained with electric warming pads. Anaesthesia was maintained with continuous intravenous infusions of ketamine (20 mg/kg/h) and xylazine (4 mg/kg/h) until the completion of the experiment. All pressures and ECG were online monitored and acquired by a calibrated special multiple-channel online recorder (MedIS, Medical Device Integration System, Hochschule Mannheim, Germany). Maintenance fluids were provided by continuous infusion of balanced electrolyte solution at a rate of 4 ml/kg/h. During instrumentation, animals were ventilated with a Fabian Plus® neonatal respirator (Acutronic Medical Systems AG, Hirzel, Switzerland) using a pressure control mode (IPPV) with a FiO_2 of 0.5, a tidal volume of 6 ml/kg, a positive end-expiratory pressure of 4 cmH_2O and a respiratory rate of 24–28 min^{-1} to achieve physiological carbon dioxide arterial partial pressures (p_aCO_2: 35–42 mmHg). Inspiratory peak pressure and respiratory rate were adjusted to maintain baseline tidal and minute volume after inhalation therapy in order to allow for detection of influences of the nebulised agents on ventilation, oxygenation and lung mechanics.

Table 2
Laser diffraction parameters, corresponding technical data and measurement conditions.

Lens	R2 (Sympatec GmbH)
Measuring range	0.25–87.5 μm
Distance between jet catheter and laser beam	2.5 cm
Measuring time for every cycle	100 s
Measuring intervals	One measurement every 20 s (5 per cycle)
Number of cycles	5
Flow rate at the nozzle tip (manufacturer information)	1.4 l/min
Driving pressure catheter	60 psi (4.1 bar)
Temperature	25 ± 1 °C
Relative humidity	49 ± 4%

2.2.2. Experimental protocol

A recruitment manoeuvre (CPAP; 10 s; PEEP 20 cm H_2O) was applied in all groups to standardize lung volume history. After a stabilization period of 15 min, baseline values were recorded. Animals were randomized to one of six experimental groups and received inhalational therapy either with saline (NaCl), a high or low dose of Perfluorohexyloctane (F6H8) or Perfluorobutylpentane (F4H5) or no inhalation (Sham). High dose was defined as 1 ml/kg bodyweight (Bw) and low dose as 0.1 ml/kg Bw. Arterial gas exchange, lung mechanics, heart rate and blood pressure were recorded prior to nebulisation (baseline) and in 30 min intervals during the 6-h study period.

Nebulisation of the test substances was performed with the aforementioned aerosolisation catheter (AeroProbe®, Trudell Medical International, Ontario, Canada) and the electromechanical catheter control system (LABneb®, Trudell Medical International, Ontario, Canada). The catheter was positioned via a sealed side port at the tip of the endotracheal tube. The control unit automatically triggered aerosolisation in synchronization with the ventilators inspiratory cycle. Total nebulisation time was registered.

Gas exchange, i.e. arterial partial pressure of oxygen, carbon dioxide and pH values, was measured with a blood gas analyzer (Cobas® B121, Roche Pharma AG, Germany). Based on results from continuous monitoring, the following measurements and calculations for lung mechanics were performed:

Total respiratory system dynamic compliance (C_{rs}):

$$\text{Dynamic } C_{rs} = \text{Vt}/(P_{peak} - \text{PEEP}_{tot}) \quad (2)$$

Total respiratory system resistance (R_{rs}):

$$R_{rs} = (P_{peak})/\text{flow rate } (\text{cm H}_2\text{O L}^{-1}\text{ s}^{-1}) \quad (3)$$

Animals were killed after the 6-h study period with an intravenous high-dose pentobarbital (100 mg/kg).

The endotracheal tube was clamped, and the thorax was carefully opened. The heart and lungs were removed en bloc from the thoracic cavity. The left lung was isolated by placing a tight loop of umbilical tape at the hilum. The right lung was flushed through the right mainstem bronchus with two aliquots of 20 ml saline. Broncho-alveolar lavage (BAL) was centrifuged to obtain residues of semifluorinated alkanes. Wet/dry ratio was determined with left apical lung samples as previously described using the gravimetric method [18].

2.3. Statistical analysis

Data analysis was performed using SAS version 9.1.3 (SAS institute, Cary, NC, USA). To analyze statistical differences at baseline, paired sample *t*-test was applied. Normally distributed values were compared among the various treatment groups by two-way repeated measurement analysis of variance (ANOVA) with all pairwise comparison procedures (Holm–Sidak method). Values are expressed as mean ± SD. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Bench study

3.1.1. Physico-chemical properties

Assuming that molecular weight and ratio of fluorinated to hydrogenated carbon atoms in a SFA molecule (RF/RH ratio) defines physico-chemical properties, we analyzed four different SFAs. As shown in Table 1, F4H5 exhibited the highest vapour pressure and lowest viscosity at moderate lipophilicity. F6H6 depicted similar viscosity and vapour pressure as F6H8 but showed the lowest lipophilicity with the lowest expected solubilising properties and was therefore excluded from the further aerosolisation tests. Presenting a high lipophilicity, F6H12 revealed the highest viscosity. F6H8 demonstrated similar lipophilicity values as F4H5. Further comparing F6H8 and F4H5 substantial differences in molecular weight (432 vs. 290 g/mol), vapour pressure (<1 vs. 25.1 torr) and viscosity (3.44 vs. 1.05 mPa) were revealed, as shown in Table 1.

3.1.2. Aerosolisation and droplet sizes

Based on the results of our bench study, output rates per minute were measured for F4H5, F6H8, F6H12 and saline at an aerosolisation pressure of 60 psi (4.1 bar). Each liquid was tested fivefold. At continuous flow rates of 1.4 l/min air (manufacturer information), output rates revealed 0.449 ± 0.023 ml/min, 0.179 ± 0.008 ml/min and 0.574 ± 0.019 ml/min for F4H5, F6H8 and saline respectively. F6H12 demonstrated output rates as low as 0.03 ± 0.004 ml/min and was therefore excluded from further aerosolisation tests. Mass median aerodynamic diameter (MMAD) of generated aerosols ranged between 2.98 and 7.43 μm (Table 3). The distribution of droplet sizes less than 5 μm was higher for F4H5 (89%) and F6H8 (88.5%) than for NaCl (40.5%). Droplet size distribution sums are shown in Fig. 1 as mean values for a total of 20 measurements.

3.2. Animal study

All animals survived the study period. The baseline values for the measured variables were comparable between groups. Throughout the experimental period, heart rate, arterial pressure and central venous pressure revealed no significant differences between the groups (data not shown). Comparisons for gas exchange and pulmonary mechanics were made between high-dose SFA groups, saline group (NaCl) and control (Sham) group, and between low-dose SFA groups and Sham, respectively.

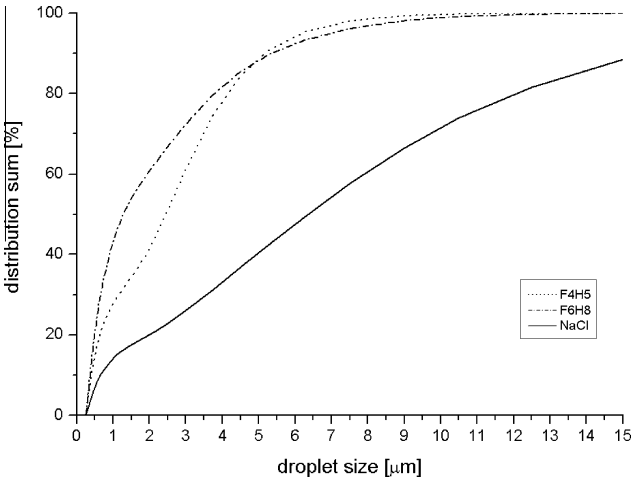

Fig. 1. Representative droplet size and distribution sum of F6H8, F4H5 and saline.

3.2.1. Gas exchange

P_aO_2/F_iO_2 ratio in all high-dose groups immediately decreased after starting aerosol therapy (Fig. 2). No differences between the high-dose groups were detected ($F4H5_{high}$ vs. $F6H8_{high}$: $p = 0.648$; $F6H8_{high}$ vs. NaCl: $p = 0.089$; $F4H5_{high}$ vs. NaCl: $p = 0.216$). Specifically in low-dose groups, there was a sustained decrease in p_aO_2/F_iO_2 ratio after $F6H8_{low}$ inhalation ($F6H8_{low}$ vs. $F4H5_{low}$: $p < 0.001$; $F6H8_{low}$ vs. Sham: $p < 0.001$). Regarding $F4H5_{low}$ group, no changes in oxygenation were seen. This group behaved like the untreated Sham-group ($F4H5_{low}$ vs. Sham: $p = 0.230$; ns) (Fig. 2).

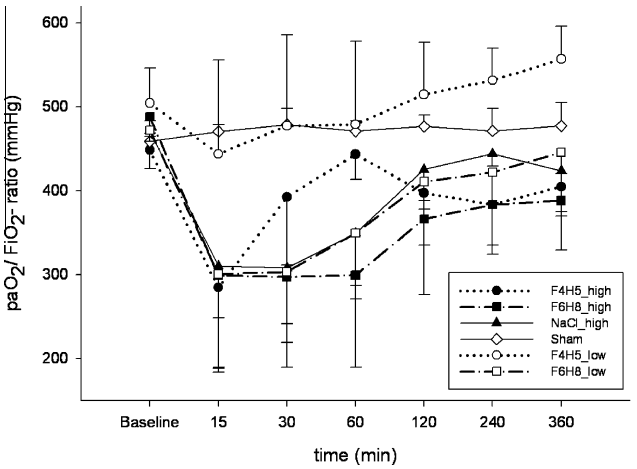

Fig. 2. paO_2/FiO_2 ratio. Oxygenation in high-dose groups (filled symbols) expressed as paO_2/FiO_2 ratio (Horowitz-index) in healthy animals at BL and after inhalation therapy during 360 min of conventional mechanical ventilation. Two-way RM ANOVA with multiple comparison procedure (Holm–Sidak method) indicated (1) significant decrease in all groups ($p < 0.05$) (b) fastest return to Sham-like values for F4H5 (30 min), followed by NaCl (60 min) and F6H8 (120 min) (c) high-dose SFA treated-groups equal effects of NaCl. Oxygenation in low-dose groups (open symbols) expressed as paO_2/FiO_2 ratio in healthy animals at BL and after inhalation therapy during 360 min of conventional mechanical ventilation. Values are mean ± SE. Two-way RM ANOVA with multiple comparison procedure (Holm–Sidak method) indicated (1) significant decrease in $F6H8_{low}$ for 60 min ($p = 0.002$) (2) no significant difference of $F4H5_{low}$ vs. Sham-groups.

Table 3
Droplet size measurement of nebulized Perfluorohexyloctane (F6H8), Perfluorobutylpentane (F4H5) and saline with Laserdiffractometer (HELOS H2109, Sympatec GmbH, Clausthal-Zellerfeld, Germany). Aerosolisation was performed with: AeroProbe®-catheter and LabNeb®-catheter control system (Trudell Medical International, Ontario, Canada). MMAD: mass median aerodynamic diameter. Data are shown as mean for a total of 20 measurements.

	F4H5	F6H8	Saline
VMD (μm)	2.64	2.24	7.43
MMAD (μm)	3.24	2.98	7.46
Droplets			
⩽5 μm (%)	89	88.5	40.5
⩽1 μm (%)	28	43	14.5

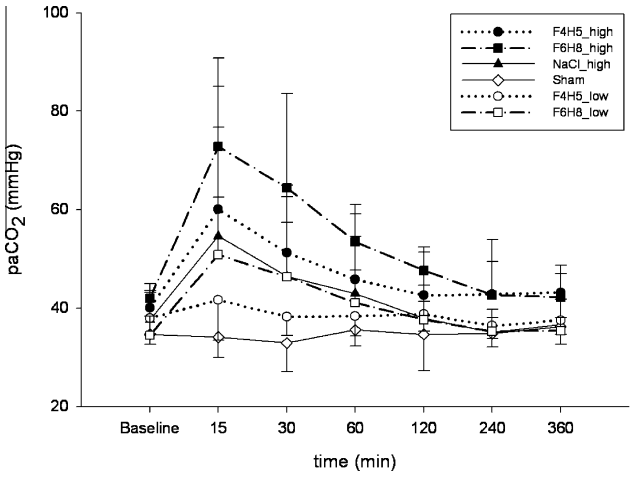

Fig. 3. Comparison of partial pressure of carbon dioxide ($PaCO_2$) for high-dose groups (filled symbols) and low-dose groups (open symbols). Differences were observed only for F6H8 (line-dot-line). ANOVA comparisons were performed with multiple comparison procedure (Holm–Sidak method) and indicated higher values for $F6H8_{high}$ vs. Sham ($p < 0.001$), for $F6H8_{high}$ vs. $NaCl_{high}$ ($p = 0.002$) and for $F6H8_{low}$ vs. Sham ($p = 0.002$). In F4H5 groups (dots), no differences to NaCl or Sham were observed. Values are mean ± SE.

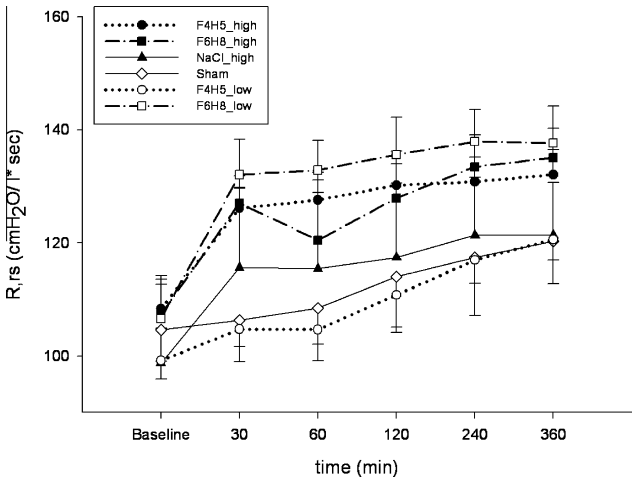

Fig. 5. Resistance of the respiratory system (R_{rs}) in high-dose groups (filled symbols) and low-dose groups (open symbols) obtained at baseline (before inhalation therapy and 15 min after standardized recruitment manoeuvre). ANOVA analysis of high-dose groups indicated a significant increase in both SFA groups. $F4H5_{low}$ equals effect of Sham-group ($p = 0.587$; not significant), while $F6H8_{low}$ shows a significant increase after nebulisation remaining significantly higher throughout the study period ($F6H8_{low}$ vs. Sham: $p < 0.001$).

$F4H5_{high}$ inhalation showed no impact on P_aCO_2 values compared to Sham, NaCl and also $F6H8_{high}$ (Fig. 3). A high-dose application of $F6H8_{high}$ transiently resulted in higher P_aCO_2 values compared to Sham ($p < 0.001$) and NaCl ($p = 0.002$). Application of SFAs after nebulisation showed no effect on arterial pH (data not shown).

3.2.2. Respiratory system mechanics

Respiratory system total dynamic compliance (C_{rs}) related to kg body weight, for high-dose groups and for low-dose groups is shown in Fig. 4. In all groups, including Sham, C_{rs} progressively decreased over time (baseline vs. time within-group $p < 0.001$). Comparing high-dose SFA with untreated Sham-group revealed differences for C_{rs}. This deterioration persisted for 120 ($F6H8_{high}$) to 180 ($F4H5_{high}$) minutes. No differences were detected between SFA_{high} and NaCl. A high discrepancy was observed in the low-dose application of the two semifluorinated alkanes ($F4H5_{low}$ vs. $F6H8_{low}$, $p < 0.001$); While C_{rs} in $F6H8_{low}$ showed a fast decline, C_{rs} values in $F4H5_{low}$ persisted on the starting level and showed the same slow decrease as the untreated Sham-group (Fig. 4: $F4H5_{low}$ vs. Sham; ns). No difference in compliance of the chest wall was observed between groups. Therefore, differences in total dynamic compliance (C_{rs}) were attributable to changes in lung compliance (CL) (data not shown).

Analysis of respiratory system resistance (R_{rs}) compared to Sham demonstrated a significant increase in $F6H8_{high}$, $F4H5_{high}$ and $F6H8_{low}$ ($p < 0.05$; Fig. 5). R_{rs} in $F4H5_{low}$ was equal to that of Sham-group. $F6H8_{low}$ values of respiratory system resistance increased directly after nebulisation remaining high throughout the study period.

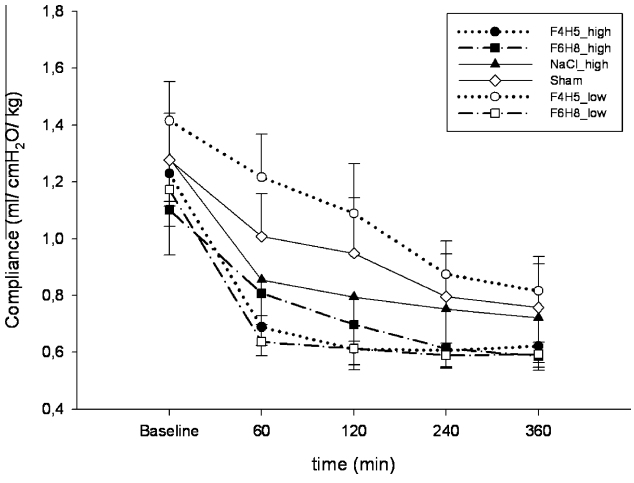

Fig. 4. Total dynamic compliance of the respiratory system (C_{rs}) in high-dose groups (filled symbols) and low-dose groups (open symbols) obtained at baseline (before inhalation therapy and 15 min after standardized recruitment manoeuvre). Values are mean ± SE. ANOVA comparisons were performed with multiple comparison procedure (Holm–Sidak method) and indicated for high-dose groups (1) both SFA vs. Sham-groups revealed significant differences for $F6H8_{high}$ ($p = 0.006$; for 120 min) and $F4H5_{high}$ ($p = 0.005$; for 180 min), (2) no significant differences between SFA_{high} and NaCl; for low-dose groups: (3) $F6H8_{low}$ – values show a fast decline with a highly significant difference to $F4H5_{low}$ and Sham-group, (4) $F4H5_{low}$ decreases slow overtime like Sham-group ($p = 0.078$; not significant).

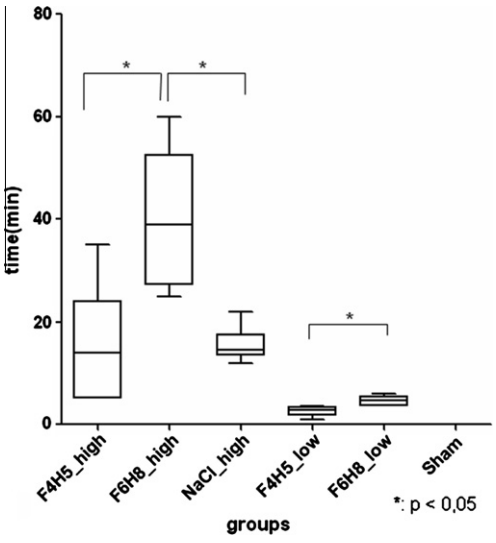

Fig. 6. Total time needed for nebulisation during the animal study. $F4H5_{high}$ vs. $F6H8_{high}$: $p < 0.05$; $F6H8_{high}$ vs. NaCl: $p < 0.05$; $F4H5_{low}$ vs. $F6H8_{low}$: $p < 0.05$.

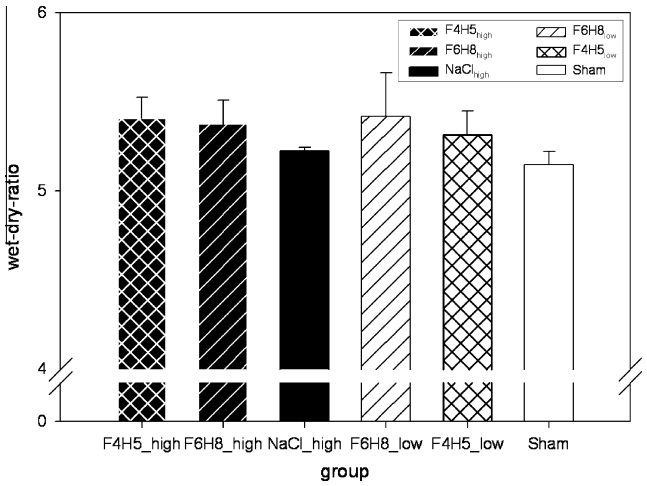

Fig. 7. Wet/dry ratio of the left lung revealed no differences between high- and low-dose SFA, saline, Sham-groups.

Total time of nebulisation in high-dose groups showed differences for $F6H8_{high}$ (40 ± 14 min) compared to $F4H5_{high}$ (16 ± 11 min; $p < 0.05$) and $NaCl_{high}$ (16 ± 3 min; $p < 0.05$), $F4H5_{low}$ application (3 ± 1 min) took significant shorter than $F6H8_{low}$ (5 ± 1 min; $p < 0.05$) (Fig. 6).

As shown in Fig. 7, no differences in wet/dry ratio between groups were detected.

After centrifuging broncho-alveolar lavage, we could detect F6H8 residues in both groups ($F6H8_{high > low}$) but not in F4H5 groups. This measurement was of qualitative nature.

4. Discussion

In the present combined bench and animal study, physico-chemical properties of four different semifluorinated alkanes (SFAs) were determined. Based on these results, we selected two SFAs with satisfactory lipophilicity, whilst concurrently being suitable for aerosolisation: Perfluorohexyloctane (F6H8) with a high viscosity and a low vapour pressure, in contrast to Perfluorobutylpentane (F4H5) with low viscosity and high vapour pressure. The in vivo effects of these two nebulised SFAs (F4H5 and F6H8) were tested by exploring oxygenation and lung mechanics in healthy rabbits to address their potential use as novel excipients for inhalative liquid drug carrier systems. The confirmatory animal study showed that a low-dose application (0.1 ml/kg) of F4H5 equals values of untreated animals (Sham) throughout the study period of 6 h; side effects were detected neither on oxygenation nor on lung mechanics and hemodynamics.

4.1. Physico-chemical properties

Intrapulmonary deposition of PFCs, either applied as a liquid or as an aerosol, seems to be dependent of their physico-chemical properties, especially vapour pressure and viscosity [12]. This affects elimination, evaporation and distribution kinetics but also has potential effects on oxygenation and pulmonary mechanics [12–14]. PFC elimination is proportional to the vapour pressure of the PFC, inversely proportional to molecular weight and systemic elimination is positively influenced by lipophilicity [19]. PFCs have been studied in the past for liquid-assisted ventilation [20–23], but also as vehicles for the intrapulmonary administration of drugs in several animal models [24–29]. Although the results suggested a promising novel intrapulmonary drug delivery approach, major obstacles including poor solubility of typical drug molecules and formulation instabilities in PFC still remain unsolved [30]. Varying physico-chemical properties of different PFCs, especially vapour pressure, lead to distinct evaporation rates and to a potential intrapulmonary accumulation [14]. As shown in an aerosolisation study, PFC with a low vapour pressure and a high viscosity can accumulate imitating liquid ventilation [13]. In a healthy lung model, this may negatively influence gas exchange and lung mechanics [15].

There are discrepant data on PFC inhalation, vapour pressure and the correlation on gas exchange and lung mechanics. In a comparison of different inhaled PFCs in a saline wash-out model, Meinhardt et al. could only detect an effect on gas exchange and lung mechanics for perfluorohexane, a PFC with high vapour pressure at high doses of 25 ml/h/kg body weight [31]. Interestingly, in another study, Kandler et al. stated that PFC–aerosol treatment improved gas exchange in lung injured piglets independent from molecular structure and vapour pressure. These findings were clearly dose dependent with an improvement in gas exchange for high-dose groups, but without any improvement for a "low dose" of 1.25 ml/kg/h [32].

After centrifuging broncho-alveolar lavage, liquid residues were detectable in high-dose F6H8 group, but not in F4H5 groups. F6H8 seems to accumulate undesirably in alveolar and bronchial structures. According to PFC data, we therefore conclude that vapour pressure might play an important role in intrapulmonary elimination kinetics after SFA-lung application. F4H5 compared to F6H8 might evaporate much faster due to its higher vapour pressure and lower molecular weight.

Vapour pressure also affects droplet lifetime after aerosolisation within the inspiratory gas flow. Droplet size changes during the passage through the airways; a higher vapour pressure may cause smaller droplets reaching deeper lung regions [13]. The present catheter system, as described previously for PFC [33], can be also used for aerosolisation of SFA with adequate droplet sizes with a MMAD about 3 μm. Droplets in the size range of 0.1–1 μm are deposited by gravitational and diffusion transport. For larger particles, like in the present study, inertial transport becomes an effective transport mechanism, and deposition due to impaction increases with particle size, particle density, and airflow rate. Thus, in the size range above 1 μm, droplets or particles are deposited due to impaction and sedimentation [34]. The distribution of droplet sizes under 5 μm was higher for SFAs than for NaCl. Hence, lung deposition for NaCl possibly occurs in larger airspaces than for F4H5 and F6H8. Smaller droplets are reaching deeper lung regions with a greater chance for alveolar deposition, but droplets with sizes near to 1 μm have a higher possibility to be exhaled. This may result in different impacts on oxygenation and respiratory system mechanics. Interestingly, the fraction of droplets <1 μm was much higher for F6H8, theoretically leading to an increased chance for exhalation. However, even in low-dose F6H8 group we could detect a significant impact on oxygenation and respiratory system mechanics compared to F4H5.

Compared to PFCs, SFAs superiorly might be more suitable as potential drug solving media regarding their lipophilic properties. The ratio between fluorinated and hydrogenated (alkylated) carbon chain defines the physico-chemical properties of SFAs. There is a clear correlation between molecular structure and lipophilic behaviour of SFAs [8,9]. A marker for lipophilic properties of SFA and PFC is the critical solution temperature in *n*-bromohexane (CST). Increasing the alkyl part of the molecule makes the compounds more lipophilic; the CST value decreases. According to this, we detected the highest lipophilicity for Perfluorohexyldodecane (F6H12) and the lowest for Perfluorohexylhexane (F6H6). Compared to the other tested SFAs, we determined the lowest solubilising behaviour for F6H6, therefore being not suitable as a potential excipient in pulmonary drug delivery systems. In contrast, F6H12 shows

low CST values with a high lipophilicity, unfortunately associated with the highest viscosity, thus being unsuitable for nebulisation in this study. Perfluorobutylpentane (F4H5) and Perfluorohexyloctane (F6H8) showed a comparable lipophilic behaviour.

Viscosity of a liquid is defined as the internal friction of fluid, which produces resistance to change in form [35]. Therefore, fluids of higher kinematic viscosity (i.e. viscosity/density) may resist aerosolisation, especially with a jet nebulizer. Whereas there is little difference in viscosity between water and F4H5 (around 1 mPa), viscosities of F6H8 and F6H12 are higher (~3 and ~6 times greater, respectively) than that of F4H5 [16]. As a result, output rates of F6H12 were unsatisfactory; but also nebulisation of F6H8 in vivo took significant longer with the present catheter system than for F4H5 or saline.

4.2. Animal study

In healthy animals, administration of PFCs in lungs showed a decrease of arterial oxygen tension and an impairment of lung mechanics in partial and total liquid ventilation, although these values were at satisfactory levels [15,21]. In the present study high-dose SFA inhalation resulted in no different oxygenation compared to saline group. A low-dose application of F4H5 showed no impact at all, even compared to Sham. F6H8 in both groups, low- and high dose, seems to interfere with oxygenation more than F4H5 in the beginning of the experiment, although at tolerable levels. A low-dose, application of saline (0.1 ml/kg) was not conducted in this study, because daily multiple inhalations with water-based drug solutions in patients with cystic fibrosis (even in children with volumes >4 ml) are considered to be without any harm on oxygenation and lung mechanics [36].

PEEP, respiratory minute volume and tidal volume were kept constant during the study. Therefore, the observed changes on lung mechanics can be basically attributed to the aerosols of saline and the two SFA. In the present study, even in untreated animals (Sham) we detected a slow decrease in total dynamic lung compliance over time (Fig. 3). We did not perform a decremental PEEP trial after the recruitment manoeuvre to determine best-PEEP in every single animal. This may lead to increasing lung atelactasis over time [37]. Before beginning inhalation therapy, we performed a standardized recruitment manoeuvre to standardize for lung volume history. Artificial increases of lung compliance could occur, as all animals were young and healthy with potential high recruitability. Differences between high-dose SFA and saline could not be detected. Nevertheless, only a low-dose application of F4H5 after inhalation therapy did not influence lung compliance and was comparable to Sham values.

Basic and inspiratory flows were kept constant throughout the study protocol. Respiratory system resistance increased after SFA high-dose application, but not after low-dose application of F4H5. Increases in lung resistance may appear after blocked airways, because of mucus or foreign bodies. Blocked bronchial airways due to droplet formation of SFA as a cause for increasing resistance is unlikely, because of physico-chemical properties with low surface and interface tensions. Wet/dry ratio revealed no significant differences between groups, and this makes edema formation after SFA application unlikely.

In the present study, aerosolisation of SFA with different physico-chemical properties showed clearly a dose-dependent impact on oxygenation and lung mechanics. The aim of the study was to detect differences in the behaviour of aerosolised SFA and the influence of different physico-chemical properties. We ascertained that based on a low-dose application of Perfluorobutylpentane (F4H5) aerosolised semifluorinated alkanes (SFA) might be suitable as potential excipients for pulmonary drug delivery in healthy lungs without side effects on gas exchange and pulmonary mechanics.

5. Conclusion

A high dose of aerosolised SFA (1 ml/kg), either F6H8 or F4H5, equals effects of high-dose inhalation of saline. Comparing low-dose SFA groups, there is a convincing discrepancy in favour of F4H5. $F6H8_{low}$ impairs pulmonary function, whereas a low-dose application of $F4H5_{low}$ shows no interference. This may be due to the faster evaporation of F4H5 in comparison with F6H8.

Perfluorobutylpentane (F4H5) in a low-dose application is suitable as a new inhalable excipient in SFA-based liquid pulmonary drug delivery systems for lipophilic or water-insoluble substances.

Acknowledgements

The authors would like to thank Dr. Christel Weiss, Department of Medical Statistics, University Hospital Mannheim, Germany for statistical advice.

Parts of the present work were presented at the Congress of the European Society of Intensive Care Medicine 2009 (ESICM) in Vienna.

This study was partially supported by Novaliq GmbH, Heidelberg, Germany.

References

[1] J.S. Patton, P.R. Byron, Inhaling medicines: delivering drugs to the body through the lungs, Nat. Rev. Drug Discov. 6 (1) (2007) 67–74.
[2] B. Tang et al., Development of solid self-emulsifying drug delivery systems: preparation techniques and dosage forms, Drug Discov. Today 13 (13-14) (2008) 606–612.
[3] R.N. Gursoy, S. Benita, Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs, Biomed. Pharmacother. 58 (3) (2004) 173–182.
[4] R.J. Shaw, Inhaled corticosteroids for adult asthma: impact of formulation and delivery device on relative pharmacokinetics, efficacy and safety, Respir. Med. 93 (3) (1999) 149–160.
[5] B. Forbes et al., Formulation of inhaled medicines: effect of delivery vehicle on immortalized epithelial cells, J. Aerosol. Med. 13 (3) (2000) 281–288.
[6] H. Meinert, A. Knoblich, The use of semifluorinated alkanes in blood-substitutes, Biomater. Artif. Cells Immobil. Biotechnol. 21 (5) (1993) 583–595.
[7] M.P. Krafft, J.G. Riess, Highly fluorinated amphiphiles and colloidal systems, and their applications in the biomedical field. A contribution, Biochimie 80 (5–6) (1998) 489–514.
[8] H. Meinert, T. Roy, Semifluorinated alkanes – a new class of compounds with outstanding properties for use in ophthalmology, Eur. J. Ophthalmol. 10 (3) (2000) 189–197.
[9] T.D. Le, R.A. Arlauskas, J.G. Weers, Characterization of the lipophilicity of fluorocarbon derivates containing halogens or hydrocarbon blocks, J. Fluorine Chem. 78 (1996) 155–163.
[10] J. Mackiewicz et al., In vivo retinal tolerance of various heavy silicone oils, Invest. Ophthalmol. Vis. Sci. 48 (4) (2007) 1873–1883.
[11] C. Tsagogiorgas et al., Semifluorierte Alkane des Typs FmHn und deren Eigenschaft als inhalativ applizierbare Medikamententräger am Beispiel von Ibuprofen am Kaninchen, Anästh. Intensivmed. 50 (7/8) (2009) 32–33.
[12] T.F. Miller et al., Effects of perfluorochemical distribution and elimination dynamics on cardiopulmonary function, J. Appl. Physiol. 90 (3) (2001) 839–849.
[13] K. von der Hardt et al., Comparison of aerosol therapy with different perfluorocarbons in surfactant-depleted animals, Crit. Care Med. 32 (5) (2004) 1200–1206.
[14] J.L. Gabriel et al., Quantitative structure–activity relationships of perfluorinated hetero-hydrocarbons as potential respiratory media. Application to oxygen solubility, partition coefficient, viscosity, vapor pressure, and density, ASAIO J. 42 (6) (1996) 968–973.
[15] A.S. Tutuncu et al., Evaluation of lung function after intratracheal perfluorocarbon administration in healthy animals, Crit. Care Med. 24 (2) (1996) 274–279.
[16] Y.K. Kim, B. Gunther, H. Meinert, A new, heavier-than-water silicone oil:a solution of perfluorohexyloctane in polydimethylsiloxane, Eur. J. Ophthalmol. 15 (5) (2005) 627–637.
[17] A. Baydur et al., A simple method for assessing the validity of the esophageal balloon technique, Am. Rev. Respir. Dis. 126 (5) (1982) 788–791.
[18] R. Nakagawa et al., Effects of high-frequency oscillatory ventilation on oleic acid-induced lung injury in sheep, Lung 186 (4) (2008) 225–232.

[19] S.F. Flaim, Pharmacokinetics and side effects of perfluorocarbon-based blood substitutes, Artif. Cells Blood Substit. Immobil. Biotechnol. 22 (4) (1994) 1043–1054.
[20] J.P. Meinhardt, M. Quintel, R.B. Hirschl, Development and application of a double-piston configured, total-liquid ventilatory support device, Crit. Care Med. 28 (5) (2000) 1483–1488.
[21] B.P. Fuhrman, P.R. Paczan, M. DeFrancisis, Perfluorocarbon-associated gas exchange, Crit. Care Med. 19 (5) (1991) 712–722.
[22] J.S. Greenspan et al., Liquid ventilation of human preterm neonates, J. Pediatr. 117 (1 Pt 1) (1990) 106–111.
[23] C.L. Leach et al., Partial liquid ventilation with perflubron in premature infants with severe respiratory distress syndrome. The LiquiVent Study Group, New Engl. J. Med. 335 (11) (1996) 761–767.
[24] M.R. Wolfson, J.S. Greenspan, T.H. Shaffer, Pulmonary administration of vasoactive substances by perfluorochemical ventilation, Pediatrics 97 (4) (1996) 449–455.
[25] T.H. Shaffer et al., Perfluorochemical liquid as a respiratory medium, Artif. Cells Blood Substit. Immobil. Biotechnol. 22 (2) (1994) 315–326.
[26] A.B. Cullen et al., Intra-tracheal delivery strategy of gentamicin with partial liquid ventilation, Respir. Med. 93 (11) (1999) 770–778.
[27] E.W. Dickson et al., Liquid ventilation with perflubron in the treatment of rats with pneumococcal pneumonia, Crit. Care Med. 30 (2) (2002) 393–395.
[28] A.R. Franz et al., Pulmonary administration of perfluorodecaline – gentamicin and perfluorodecaline – vancomycin emulsions, Am. J. Respir. Crit. Care Med. 164 (9) (2001) 1595–1600.
[29] C. Brun-Buisson, F. Lemaire, Administration of antibiotics for pneumonia during respiratory failure: reaching the target, Am. J. Respir. Crit. Care Med. 164 (9) (2001) 1554–1555.
[30] H.J. Lehmler, Perfluorocarbon compounds as vehicles for pulmonary drug delivery, Expert. Opin. Drug Deliv. 4 (3) (2007) 247–262.
[31] J.P. Meinhardt et al., Comparison of different inhalational perfluorocarbons in a rabbit model of acute lung injury, ASAIO J. 51 (1) (2005) 85–91.
[32] M.A. Kandler et al., Dose response to aerosolized perflubron in a neonatal swine model of lung injury, Pediatr. Res. 56 (2) (2004) 191–197.
[33] M.A. Kandler et al., Persistent improvement of gas exchange and lung mechanics by aerosolized perfluorocarbon, Am. J. Respir. Crit. Care Med. 164 (1) (2001) 31–35.
[34] J. Heyder, Deposition of inhaled particles in the human respiratory tract and consequences for regional targeting in respiratory drug delivery, Proc. Am. Thorac. Soc. 1 (4) (2004) 315–320.
[35] J. Weast, C. Johnson, CRC Handbook of Chemistry and Physics, 66th ed., CRC, Boca Raton (FL), 1986.
[36] M.R. Elkins et al., A controlled trial of long-term inhaled hypertonic saline in patients with cystic fibrosis, New Engl. J. Med. 354 (3) (2006) 229–240.
[37] B.P. Kavanagh, Perioperative atelectasis, Minerva Anestesiol. 74 (6) (2008) 285–287.