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Aerosolized semifluorinated alkanes as excipients are suitable for inhalative drug delivery—A pilot study

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ABSTRACT

Semifluorinated alkanes (SFAs) have been described as potential excipients for pulmonary drug delivery, but proof of their efficacy is still lacking. We tested whether SFA formulations with the test drug ibuprofen can be nebulised and evaluated their pharmacokinetics.

Physico-chemical properties of five different ibuprofen formulations were evaluated: an aqueous solution (H2O), two different SFAs (perfluorohexyloctane (F6H8), perfluorobutylpentane (F4H5)) with and without ethanol (SFA/EtOH). Nebulisation was performed with a jet catheter system. Inhalative characteristics were evaluated by laser diffraction. A confirmative animal study with an inhalative single-dose (6 mg/kg) of ibuprofen with each formulation was performed in anaesthetised healthy rabbits. Plasma samples at defined time points and lung tissue harvested after the 6-h study period were analyzed by HPLC–MS/MS. Pharmacokinetics were calculated using a non-compartment model.

All formulations were nebulisable. No differences in aerodynamic diameters (MMAD) were detected between SFA and SFA/EtOH. The ibuprofen plasma concentration–time curve (AUC) was highest with F4H5/EtOH. In contrast, F6H8/EtOH had the highest deposition of ibuprofen into lung tissue but the lowest AUC.

All tested SFA and SFA/EtOH formulations are suitable for inhalation. F4H5/EtOH formulations might be used for rapid systemic availability of drugs. F6H8/EtOH showed intrapulmonary deposition of the test drug.

1. Introduction

Inhaled aerosols have been used since ancient times for direct drug delivery into the diseased lung (Anderson, 2005; Rau, 2005). In the past two decades, the medical establishment has started to recognize the significant potential of this route for non-invasive systemic administration of therapeutics (Patton et al., 2004). Novel therapeutic approaches evolved, e.g. inhalative insulin and gene replacement therapy or patient-specific targeting of inhaled drugs (Patton and Byron, 2007). About 40% of new drug candidates display formulation problems related to low solubility in water or high lipophilicity (Gursoy and Benita, 2004; Tang et al., 2008). Despite the rapid technological progress in inhalational medicine, the choice of excipients used to increase the solubility of hydrophobic or lipophilic drugs in an aqueous medium for pulmonary application is unfortunately very limited (O'Riordan, 2002; Pilcer and Amighi, 2010).

Semifluorinated alkanes (SFAs) are colourless liquids with fluorocarbon (R_F) and hydrocarbon (R_H) segments and, similar to perfluorocarbons (PFCs), are considered to be extremely hydrophobic, and also thermally, chemically and biologically inert (Krafft and Riess, 1998; Meinert and Knoblich, 1993). Although both substance classes show high physical solubility for oxygen and carbon dioxide, PFCs are poor solvents for typical drug molecules (Lehmler et al., 2008; Patrick, 1971). In contrast to PFCs, SFAs are amphiphilic compounds given by the lipophobic R_F-segment and the lipophilic R_H-segment (Le et al., 1996; Meinert and Roy, 2000). As indicated previously, SFAs have the potential to be used as solvents

Abbreviations: F4H5, perfluorobutylpentane; F6H8, perfluorohexyloctane; GSD, geometric standard deviation; MMAD, mass median aerodynamic diameter; PFC, perfluorocarbon; SFA, semifluorinated alkane.

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for selected drugs in their non-ionized, physiologically active form (Meinert and Roy, 2000). Recently, two SFAs, perfluorobutylpentane (F4H5) and perfluorohexyloctane (F6H8) were identified as potential excipients for inhalative drug delivery (Tsagogiorgas et al., 2010). Furthermore, the effectiveness of pulmonary drug deposition with rapid systemic absorption of ibuprofen was demonstrated in an experimental model of partial liquid ventilation (PLV) with F6H8 (Dembinski et al., 2010).

To our knowledge SFAs have not yet been used as inhalative or aerosolized drug carriers. As proposed for PFCs by Lehmler et al. (Lehmler et al., 2008), various SFAs might have different drug release properties. We hypothesized that SFAs are suitable as excipients for inhalative drug delivery and that the physico-chemical properties might not only influence lung mechanics and oxygenation, as recently shown (Tsagogiorgas et al., 2010), but may also differ in the deposition of aerosolized drugs. Since ethanol (EtOH) is soluble in alkanes as well as in SFAs (Napoli et al., 1997, 2001) it could be used as a co-solvent to increase drug concentrations in SFAs. To test this we studied the aerosolization characteristics of these formulations with regard to their suitability for inhalative purposes. We then conducted animal studies to confirm the feasibility of inhalative drug delivery using SFAs as excipients with and without EtOH. Plasma and tissue concentrations of the model drug ibuprofen were quantified to evaluate pharmacokinetics and to demonstrate the effects of F4H5 and F6H8 with and without EtOH on systemic delivery and drug deposition in healthy rabbit lungs.

2. Methods

The 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.

2.1. Bench studies

2.1.1. Chemicals

All chemical reagents were purchased from Sigma–Aldrich (Sigma–Aldrich Chemie GmbH, Munich, Germany) unless otherwise indicated. Formulations of ibuprofen in H2O, SFA and SFA/EtOH were obtained prepared for inhalation from Novaliq GmbH, Heidelberg. Two different SFAs suitable for aerosolization, perfluorohexyloctane (F6H8) and perfluorobutylpentane (F4H5) (Tsagogiorgas et al., 2010) were used. The ethanol concentration in the SFA/EtOH formulations is given as 5% (v/v). The ibuprofen concentrations in the formulations are given in Table 1. An aqueous solution of ibuprofen (6 mg/ml) served as a control. All formulations were clear solutions without any precipitations.

2.1.2. Measurement of physico-chemical properties

Density was determined at 25 °C by the oscillating body method (DA-100 M, Mettler-Toledo GmbH, Germany). Surface tension was measured at 25 °C by the Du Noüy-ring method (DCAT11, dataphysics Instruments GmbH, Germany). Kinematic viscosity was determined at 25 °C by repeated measurements with an Ubbelohde-type viscometer (Schott GmbH, Germany). Dynamic viscosity was calculated as described previously (Tsagogiorgas et al., 2010). The refractive index was determined at 20 °C with an Abbe-refractometer (AR6D, Krüss-Optronic GmbH, Germany).

2.1.3. Nebulisation and evaluation of droplet sizes

The test substances were nebulised with a nebulising catheter (AeroProbe, Trudell Medical International, Ontario, Canada) and an electromechanical catheter control system (LABneb, Trudell Medical International, Ontario, Canada) at air-conditioned ambient temperature. The catheter was advanced through a sealed side-port connector and positioned at the tip of an endotracheal tube. The control unit triggered nebulisation in synchronization with the inspiratory cycle of the ventilator (Fabian Plus®, Acutronic Medical, Switzerland). The catheter is capable of a high output rate at continuous nebulisation (>0.5 ml/min). The volume mean diameter (VMD in μm), cumulative distribution sum and geometric standard deviation (GSD) of the nebulised SFA, SFA/EtOH and the aqueous (H2O) ibuprofen solutions were measured with a laser diffraction particle sizer (HELOS H2109, Sympatec GmbH, Clausthal-Zellerfeld, Germany) under comparable flow and pressure conditions (Hinds, 1999). Data were evaluated by Sympatec Windox 5.3.1.0-Software using the MIE-theory with a shape factor of 1 (1 = spherical particle). The refractive index of each solution was included in the calculations. The mass median aerodynamic diameter (MMAD), as a marker for the deposition efficiency, was calculated according to the following equation:

$$\mathrm{MMAD} = \mathrm{VMD}\sqrt{\frac{\rho_\mathrm{p}}{\rho_0 \chi}} \quad (1)$$

assuming spherical particles with a shape factor $\chi = 1$ and density $\rho_0 = 1\ \mathrm{g/cm^3}$ (de Boer et al., 2002; Hinds, 1999; Pilcer and Amighi, 2010) and density ρ_b ($\mathrm{g/cm^3}$) for each tested formulation.

2.2. Animal study

2.2.1. Animal preparation and monitoring

Thirty-five New Zealand White rabbits weighing 2.6 ± 0.13 kg were anaesthetised with ketamine (25 mg/kg, i.m., Ketanest 10%®, Pfizer, Karlsruhe, Germany) and xylazine (5 mg/kg i.m., Rompun®, BayerVital, Leverkusen, Germany). The anaesthetised animals were placed in the supine position, the anterior neck was dissected and a tracheotomy was performed. An uncuffed endotracheal tube (3.5–4.0 inner diameter) was placed in the trachea and secured in position. A catheter for blood pressure monitoring was inserted into the common carotid artery. A 4F three-lumen catheter (Arrow Int., Teleflex medical GmbH, Kernen, Germany) was inserted into the internal jugular vein and the tip advanced into the superior vena cava. The catheters were connected to pressure transducers and central venous and arterial blood pressures were monitored. Heart rate was monitored by ECG. Tidal volume was measured with a pneumotachograph (MIM GmbH, Krugzell, Germany) connected directly to the endotracheal tube. Pancuronium bromide (0.2 mg/kg) was given to prevent spontaneous breathing efforts and repeat doses of 0.1 mg/kg were given as necessary. Body temperature was kept constant with electric warming pads. Anaesthesia was maintained with ketamine (20 mg/kg/h) and xylazine (4 mg/kg/h) infusions until the completion of the experiment. Blood pressures and ECG were monitored online and recorded on a calibrated multiple-channel online device (MedIS, Medical Device Integration System, Hochschule Mannheim, Germany). A balanced electrolyte solution was infused at a rate of 4 ml/kg/h. The animals' lungs were ventilated with a Fabian Plus® neonatal respirator (Acutronic Medical Systems AG, Hirzel, Switzerland) in pressure control mode (PCV) with an 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 maintain carbon dioxide arterial partial pressures within physiological limits (p_aCO_2: 35–42 mmHg).

2.2.2. Experimental protocol

A recruitment manoeuvre (CPAP; 10 s; PEEP 20 cmH_2O) was performed in all animals to standardize lung volume history and to reduce atelectatic lung regions. The animals were randomized to one of five experimental groups to receive inhalational ibuprofen dissolved in 1. H2O, 2. perfluorohexyloctane (F6H8), 3.

Table 1
Physico-chemical properties of the tested SFAs, Perfluorohexyloctane (F6H8) and Perfluorobutylpentane (F4H5) and ibuprofen and SFA formulations with ethanol as co-solvent (5% v/v; F6H8/EtOH and F4H5/EtOH).

	F4H5	F6H8	F4H5/EtOH	F6H8/EtOH	H2O
Ibuprofen (mg/ml)	17	6	52	48	6
Density (at 22.3 °C) (g/cm^3)	1.287	1.3335	1.2524	1.2936	1.0071
Surface tension (mN/m)	17.22 ± 0.0064	19.59 ± 0.0065	17.45 ± 0.0066	19.69 ± 0.0066	45.55 ± 0.0069
Viscosity (at 25.3 °C) (mPa s)	0.9	2.3	0.7	2	0.7
Refractive index	1.3245	1.346	1.3325	1.3515	1.3362

F6H8 with ethanol (F6H8/EtOH), 4. perfluorobutylpentane (F4H5) or 5. F4H5 with ethanol (F4H5/EtOH). Nebulisation was performed as described previously (Tsagogiorgas et al., 2010). The applied ibuprofen dose was 6 mg/kg body weight in all animals. Arterial gas exchange, heart rate and blood pressures were recorded prior to nebulisation (baseline) and at 30 min intervals during the 6-h study period.

Plasma samples were obtained after the start of nebulisation at 1, 5, 10, 15, 30, 45, 60, 120, 180, 240, 300, and 360 min, centrifuged and stored at −20 °C until ibuprofen analysis.

At the end of the study period, the animals were sacrificed in deep sedation with intravenous potassium chloride. The lungs were removed and left lung was stored at −80 °C until further processing.

2.3. HPLC–MS/MS analysis of ibuprofen

Plasma and tissue samples were analyzed by HPLC (Purospher Star, Merck KgaA, Germany) and mass spectrometry (MS/MS; API4000, Applied Biosystems, Germany). Technical data are given in Table 2.

2.3.1. Calibration standards

For calibration standards of the plasma and tissue samples ibuprofen was weighed and dissolved in methanol to a concentration of 1 mg/ml. This solution was then diluted with methanol/water (90 + 10, v/v) to a final concentration of 100 μg/ml. From this stock solution ten calibration standard solutions were made using the same solvent: 20, 50 100, 200, 500, 1000, 2000, 5000, 10,000 and 20,000 ng/ml. 10 μl of the calibration standard solutions were each pipetted into 10 ml polypropylene tubes (NUNC Immuno tubes, Nalge, USA) containing 200 μl blank rabbit lung homogenate or 100 μl blank rabbit plasma. Ten final concentrations of the calibration standards ranging from 4 to 4000 ng/g lung tissue or 2–4000 ng/ml plasma were prepared. The calibration curves were calculated by linear regression of the area under peak ratios of ibuprofen and internal standards (ANALYST software, Applied Biosystem, version 1.4.2, Carlsbad, CA, USA) and were linear over the entire concentration range (lung tissue: $y = 0.0004 \times x + 0.00126$; $r^2 = 0.9961$; plasma: $y = 0.0008 \times x - 0.00049$; $r^2 = 0.9952$, respectively). Ibuprofen concentrations were calculated from the corresponding calibration curves using ANALYST® software.

2.3.2. Quality controls

Quality control (QC) samples were prepared accordingly with final concentrations of 30, 300, 600 and 3000 ng/g lung tissue or 15, 150, 300 and 1500 ng/ml plasma. In order to ensure consistent accuracy of the measured ibuprofen concentrations, QC samples in rabbit lung homogenates or rabbit plasma were analyzed together with each analytical batch. QC samples were spiked by an independent person. One QC sample of each concentration was analyzed before the test samples and after the test samples within each analytical batch.

2.3.3. Internal standard

The internal standard ibuprofen-d_3 was dissolved in methanol to a concentration of 1 mg/ml. This solution was further diluted with methanol/water (90 + 10, v/v) to a final concentration of 10 μg/ml. An aliquot of 10 μl of this solution was spiked to each lung homogenate or plasma sample. The concentration of the internal standard in the lung tissue samples was 500 ng/ml (=2 μg/g).

2.4. Sample preparation

2.4.1. Calibrations standard and QC

Calibration standards and QC samples of lung tissue were spiked with 10 μl internal standard solution (10 μg/ml) and 200 μl solution acetonitrile and methanol (50 + 50; v/v; AM). These samples were then vortexed and stored frozen for about 5 min at approximately −40 °C and then vortexed again. Furthermore, the samples were centrifuged for 20 min at 20,000 × *g*. The upper phase was then pipetted into HPLC-vials and 20 μl of each sample was injected directly into the HPLC–MS/MS system.

2.4.2. Preparation of the lung homogenates

Mediastinal and basal lung tissue samples of rabbits were thawed and weighed. After addition of 3 ml phosphate buffer solution per 1 g lung tissue, the mixture was homogenized by using a Teflon potter. The homogenates were then subsequently portioned and immediately frozen at approximately −20 °C until analysis processing.

2.4.3. Test samples

An aliquot of 200 μl homogenate of the test samples was taken and spiked with 10 μl internal standard solution (10 μg/ml) and 200 μl solution AM. The samples were then vortexed and stored frozen for about 5 min at approximately −40 °C and then vortexed again. Afterwards, the samples were centrifuged for 20 min at 20,000 × *g*. The upper phase was then pipetted into HPLC-vials and 20 μl of each sample was injected directly into the HPLC–MS/MS system.

The limit of quantification (LOQ) of the analytical method for lung homogenates was 4 ng/g for undiluted test samples.

Fractions of 10 μl calibration standards and QC samples of rabbit plasma were spiked into 100 μl of the test samples (final concentration of ibuprofen in plasma: 200 ng/ml). Afterwards, 150 μl methanol was added. The samples were shaken and centrifuged (20,000 × *g*) for 15 min. Aliquots of 100 μl were pipetted into HPLC vials of which 50 μl were injected directly into the HPLC system. The LOQ of the analytical method for plasma samples was 2 ng/g for undiluted test samples.

2.5. Pharmacokinetic analysis

Pharmacokinetic (PK) parameters were determined by non-compartmental PK analysis using (PK Solutions 2.0, Summit Research Services, Montrose, CO, USA). These included maximal plasma concentration (Cmax), time to reach maximal

Table 2
Technical data of the HPLC–MS/MS system for ibuprofen analysis.

Mass spectrometer (Applied Biosystems, Darmstadt, Germany)	API 4000 Scan type: MRM (multiple reaction monitoring) Polarity: negative MS parameters: Internal standard Q1 mass: 207.9 amu Q3 mass: 160.9 amu Ibuprofen Q1 mass: 205.0 amu Q3 mass: 158.8 amu
HPLC-column (Merck, Darmstadt, Germany)	Purospher Star RP 18e, 5 μm
HPLC-conditions	Flow rate: 600 μl/min Run time: 11 min Injection volume: 20 μl Column temperature: 40 °C

plasma concentration (Tmax) and the area under the plasma concentration–time curve (AUC).

2.6. Statistical analysis

Data analysis was performed with SAS® (Version 9.1.3, SAS institute, Cary, NC, USA) and SigmaPlot® (Version 11.0, Systat Software Inc., San Jose, CA, USA). Aerosol characteristics, Cmax, Tmax and values for AUC_{0-360} were compared between groups with ANOVA and Tukey's post hoc test. The values of ibuprofen concentrations in lung tissue were compared with the Kruskal–Wallis signed-rank test and Dunn's post hoc test. Statistical significance was defined as $p < 0.05$. Descriptive statistics are expressed as mean ± SD.

3. Results

3.1. Bench studies

3.1.1. Physico-chemical properties of the ibuprofen formulations

Adding ethanol (EtOH) to the SFAs did not cause any appreciable change in density, surface tension or refractive index, but the viscosity decreased (Table 1). The F4H5/EtOH formulation had demonstrated the same viscosity as the aqueous formulation.

3.1.2. Nebulisation and droplet sizes

Parameters for laser diffraction, corresponding technical data and measurement conditions are given in Table 3. It was possible to nebulise all formulations.

The cumulative distribution curves of the droplet sizes for the formulations are given in Fig. 1. The distribution sums are given as mean values for a total of eight measurements. The respirable fraction (Q3 < 5 μm) of all tested SFA and SFA/EtOH formulations was higher than that of the H2O formulation (Table 4; $p < 0.001$; H2O versus all SFA formulations). The fraction of potentially exhalable droplets (Q3 < 1 μm) was higher in F4H5/EtOH than in F4H5 ($p < 0.05$, F4H5/EtOH versus F4H5). There was no difference in this parameter between the two SFA/EtOH formulations.

The aqueous ibuprofen solution (H2O) showed the highest values for GSD and MMAD but was still suitable for bronchial and alveolar delivery (Table 4). The MMADs of all SFA and SFA/EtOH formulations were lower than that of H2O ($p < 0.001$; all SFA and SFA/EtOH formulations versus H2O). The MMAD of F4H5 was higher than that of F6H8 and both SFA/EtOH formulations (Table 4; $p < 0.05$; F4H5 versus F6H8, F6H8/EtOH and F4H5/EtOH). All SFA and SFA/EtOH formulations were suitable for inhalation. Furthermore, ethanol did not noticeably change the aerosol properties of the nebulised SFAs and is therefore suitable as a co-solvent in inhalative SFA-formulations.

3.2. Animal study

All animals survived the study period. The baseline values of the measured variables were similar in all groups. Heart rate and arterial and central venous pressures were within normal limits and did not differ significantly between the groups (data not shown).

3.2.1. Pharmacokinetics

Plasma concentrations for ibuprofen after a single-dose inhalative administration of 6 mg/kg body weight for F4H5, F6H8 and H2O groups are given in the left panel, for F4H5/EtOH and F6H8/EtOH groups in the right panel of Fig. 2. The pharmacokinetic parameters are given in Table 5. The time to maximal plasma concentration (Tmax) was shortest for F4H5/EtOH. Tmax of F4H5 and both SFA/EtOH formulations were shorter than that of H2O ($p < 0.01$; F4H5, F4H5/EtOH and F6H8/EtOH versus H2O). No differences were found between the SFA formulations. The maximal plasma concentrations (Cmax) of ibuprofen was highest for F4H5/EtOH, but only with a significant difference to F4H5 ($p < 0.05$; F4H5/EtOH versus F4H5). Highest values for plasma concentration–time curve (AUC) were achieved for F4H5/EtOH and lowest for F6H8/EtOH ($p < 0.05$; see Table 5), although MMAD as a marker for deposition efficiency and exhalable droplet fraction <1 μm were similar.

3.2.2. Residue of ibuprofen in lung tissue

To further clarify the fate of ibuprofen in F6H8/EtOH after nebulisation we analyzed ibuprofen in lung tissue. Interestingly, the analysis of basal and mediastinal lung lobes revealed the highest concentration for ibuprofen in F6H8/EtOH after 6 h (see Fig. 3; $p < 0.05$; F6H8/EtOH versus F4H5).

4. Discussion

In the present study we determined the physico-chemical properties of inhalative ibuprofen formulations using two different SFAs with and without ethanol (EtOH) as a co-solvent and evaluated the inhalative characteristics.

A follow-up animal study was performed to determine whether SFAs as inhalative excipients are capable of transporting drugs into the lungs and whether there are differences between the types of SFA.

The main results of the in vitro study are: (1) All formulations are suitable for nebulisation. (2) The tested SFA/EtOH-formulations had comparable nebulisation properties. (3) In the animal study, we observed that detectable systemic drug concentrations were achieved with all tested ibuprofen formulations. (4) Systemic availability of ibuprofen was highest for F4H5/EtOH, whereas (5)

Table 3
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	23.4 ± 1.3 °C
Relative humidity	49 ± 4%

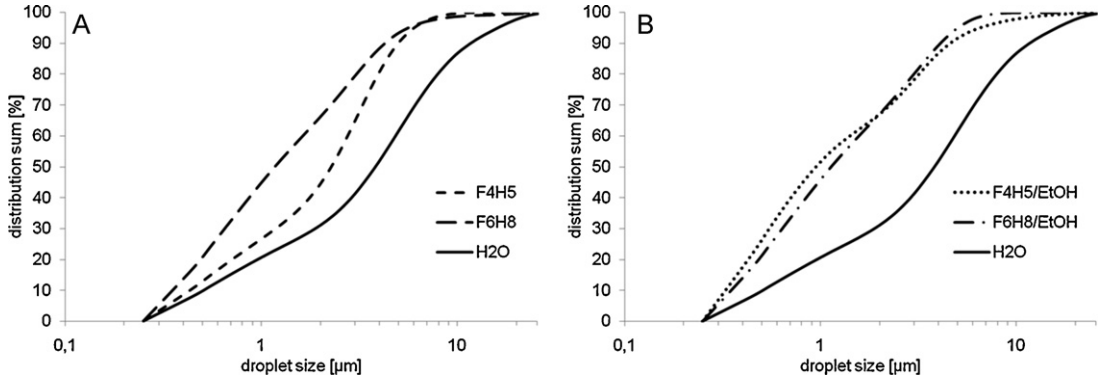

Fig. 1. Cumulative distribution curve (logarithmic size scale). Droplet size distribution of aerosolized semifluorinated alkanes (SFA), perfluorobutylpentane (F4H5) and perfluorohexyloctane (F6H8) with ibuprofen (A) in comparison to a water-based ibuprofen solution (H2O). Droplet size distribution of aerosolized SFA formulations with ibuprofen with co-solvent ethanol (EtOH; 5 vol.%) (B).

Table 4
Droplet size distribution and values of mass median aerodynamic diameter (MMAD), volume mean diameter (VMD), geometric standard deviation (GSD) and inhalable fraction with distribution sum of droplets under 1 or 5 μm (Q3 (1 μm) and Q3 (5 μm)).

	F4H5	F6H8	F4H5–EtOH	F6H8–EtOH	H2O
GSD	2.71 ± 0.07	2.82 ± 0.05	3.11 ± 0.43[#]	2.8 ± 0.02	3.48 ± 0.06[*]
VMD (μm)	2.53 ± 0.21	1.98 ± 0.21[#]	2.04 ± 0.53[#]	1.76 ± 0.04	5.17 ± 0.48[*]
MMAD (μm)	2.87 ± 0.23	2.29 ± 0.24[#]	2.28 ± 0.59[#]	1.99 ± 0.06[#]	5.18 ± 0.48[*]
Q3 (1 μm) (%)	26.59 ± 1.70	44.72 ± 0.55[#,§]	51.31 ± 7.10[#,§]	45.73 ± 0.73[#,§]	20.52 ± 1.67
Q3 (5 μm) (%)	89.97 ± 2.86	93.25 ± 1.41	91.44 ± 6.33	94.88 ± 0.64	61.42 ± 4.24[*]

All values are given as mean ± SD.
[*] $p < 0.001$, H2O versus all SFA ± EtOH.
[#] $p < 0.05$, versus F4H5.
[§] $p < 0.05$, versus H2O.

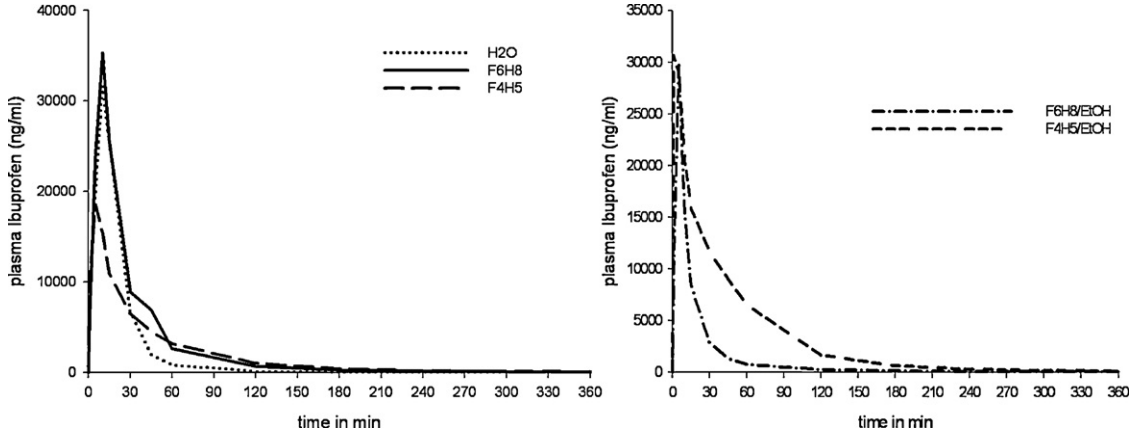

Fig. 2. Plasma concentration of ibuprofen after single-dose inhalative administration. The ibuprofen dose in all groups was 6 mg/kg bodyweight. Ibuprofen was delivered with nebulised SFA (F4H5, F6H8) or H2O (left panel) and nebulised SFAs with co-solvent ethanol (F4H5/EtOH and F6H8/EtOH; right panel).

Table 5
Single-dose pharmacokinetics of ibuprofen after inhalative administration.

Group	Dose (mg/kg)	Cmax (μg/ml)	AUC_{0-360} (μg/ml min)	Tmax (min)
H2O	6	33 ± 9.9	698.0 ± 276.9	10.8 ± 2.0
F6H8	6	37.7 ± 15.3	942.5 ± 541.1	9 ± 2.2
F4H5	6	21.1 ± 7.1	680.7 ± 215.2	6.4 ± 2.4*
F6H8–EtOH	6	30.2 ± 9.9	440.8 ± 100.6	5.7 ± 1.9*
F4H5–EtOH	6	42.1 ± 11.7#	1191.4 ± 592.9§	3.4 ± 2.2*

Values are presented as arithmetic means ± SD. AUC: area under the drug concentration time curve; Cmax: maximum observed plasma drug concentration; Tmax: time to maximum plasma drug concentration; and min: minutes.
* $p < 0.01$, versus H2O.
# $p < 0.05$ versus F4H5.
§ $p < 0.05$, versus F6H8–EtOH.

F6H8/EtOH had the lowest AUC with an increased accumulation of ibuprofen in the lung tissue after 6 h.

4.1. PFC and SFA as drug carriers in liquid ventilation

In the past decades, PFCs have been extensively studied for liquid-assisted ventilation as a therapeutic approach in acute lung injury and acute respiratory distress syndrome (ALI/ARDS) (Fuhrman et al., 1991; Greenspan et al., 1990; Leach et al., 1996; Meinhardt et al., 2000). However, research activities decreased after a multicenter study failed to show a beneficial effect of PLV in ARDS patients (Kacmarek et al., 2006). Nevertheless, the use of PFC for drug delivery was conceptually attractive especially when shunting of blood flow leads to poor lung penetration and limits therapy (Lehmler, 2007). Therefore, PFCs were further evaluated as vehicles for pulmonary drug delivery in various formulations, e.g. dispersions and emulsions, in several animal models (Brun-Buisson and Lemaire, 2001; Cullen et al., 1999; Dickson et al., 2002; Franz et al., 2001; Shaffer et al., 1994; Wolfson et al., 1996). The results were encouraging at first sight, but the poor solubility of typical drug molecules and formulation instabilities in PFCs are still limiting factors for this promising method of intrapulmonary drug delivery (Lehmler, 2007). Perfluorohexyloctane (F6H8) was recently shown to be beneficial as a liquid oxygen-carrier in PLV and might be used for drug targeting in acute respiratory failure (Dembinski et al., 2010). This might open a new chapter in partial or total liquid ventilation, delivering not only oxygen but also anti-inflammatory drugs to the injured lung with a liquid medium at the same time.

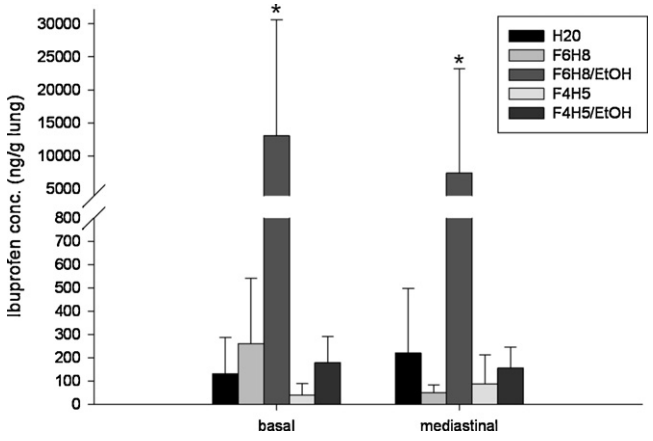

Fig. 3. Lung tissue ibuprofen concentration 6 h after single-dose inhalative administration (6 mg/kg bw). Highest concentrations could be demonstrated for perfluorohexyloctane with ethanol (F6H8/EtOH). Values are presented as arithmetic means ± SD (F6H8/EtOH versus F4H5; *$p < 0.05$; Kruskal–Wallis-test; Dunn's post test).

4.2. Influence of physico-chemical properties of nebulised PFC and SFA

In the past, nebulised PFCs have also been used to treat ALI and ARDS with promising results, improving oxygenation and lung mechanics in a dose-dependent manner (Bleyl et al., 1999; Kandler et al., 2004, 2001), and attenuating inflammation in experimental acute lung injury (Bleyl et al., 2010a,b). The physico-chemical properties of the PFCs such as vapour pressure and specific density seem to contribute to the differences in their nebulisation (Rudiger et al., 2004). Furthermore, the varying vapour pressures of different PFCs lead to distinct evaporation rates and to a potential for intrapulmonary accumulation (Gabriel et al., 1996) that would imitate liquid ventilation (von der Hardt et al., 2004). Recently, Murgia et al. suggested that kinematic viscosity might also play an important role in the nebulisation behavior of different PFCs using the same catheter system as in the present study (Murgia et al., 2011).

Lately, the physico-chemical and aerosol properties have been evaluated to test the suitability of different SFAs as excipients in inhalative drug delivery (Tsagogiorgas et al., 2010). As with PFC nebulisation, the authors concluded that vapour pressure and kinematic viscosity are important factors influencing SFA nebulisation and intrapulmonary elimination kinetics. Nebulised F6H8, an SFA with lower vapour pressure and higher viscosity than F4H5, in higher doses seems to accumulate in alveolar and bronchial structures at higher doses (Tsagogiorgas et al., 2010) thus imitating liquid ventilation (von der Hardt et al., 2004) and altering lung mechanics and oxygenation in the healthy lung similar to PFC (Tsagogiorgas et al., 2010; Tutuncu et al., 1996). The evaporation rate of F4H5 after deposition might be higher than F6H8 due to its higher vapour pressure and lower molecular weight (Tsagogiorgas et al., 2010).

4.3. Droplet size and drug delivery of inhalative SFA formulations

The droplet size distribution and MMAD are key parameters for estimating the deposition characteristics in the lungs and the bioavailability of inhaled drugs (Pilcer et al., 2008; Ziegler and Wachtel, 2005). The optimal droplet size for pulmonary deposition is thought to be between 1 and 5 μm (Bisgaard et al., 2002; Heyder et al., 1986). Droplets in the range of 0.1–1 μm are deposited by gravitational and diffusion transport, but also have a greater probability of being exhaled. The present catheter system was described previously and can be used for nebulisation of PFCs (Kandler et al., 2001; Murgia et al., 2011) and SFAs (Tsagogiorgas et al., 2010) giving adequate MMADs about 2–3 μm.

There are two principles for measuring droplet size distribution. The impaction and impinger method is the current "gold standard" for inhaler testing, but the process of aerosol analysis is time consuming and very labor intensive (Ziegler and Wachtel, 2005). Laser diffraction techniques have become the most widely used method. They are considered fast alternative (Bisgaard et al., 2002;

Pilcer et al., 2008) and provide meaningful results, at least for solution formulations (Clark, 1995). Initially, laser diffraction measures geometrical and not aerodynamic particle size without considering particle density or shape factor (Mitchell and Tservistas, 2006). However, for liquid droplets where the density is known and the dynamic shape factor is close to the values for pure water (Clark and Borgström, 2002), aerodynamic diameter can be calculated (Crowder et al., 2002; de Boer et al., 2002; Hinds, 1999).

Another consideration to be taken into account is that droplet evaporation during laser diffraction is less relevant (de Boer et al., 2002). If the droplets contain volatile components, such as F4H5, with a high vapour pressure (Tsagogiorgas et al., 2010) they will evaporate in the air stream, and the size distribution will change as a function of the travelled distance. This is an aspect that cannot be studied with impactors and liquid impingers (Ho et al., 1986; Ranucci, 1992). It is interesting to note that there were no detectable differences in the in vitro nebulisation properties (i.e. MMAD, droplet size distribution sums and exhalable fractions) of the two SFA/EtOH formulations. The in vivo drug deposition was therefore expected to be nearly equal in both groups, since MMAD is considered as deposition marker. However these formulations showed striking differences in their drug delivery behavior. In the present study, nebulisation was performed in the trachea under comparable flow and ventilation parameters. We assumed that in vivo droplet sizes were correlated to the in vitro measurements taking into account that the ambient temperature was higher in the animals, and that evaporation was thus potentially increased. This could lead to smaller droplets in the F4H5-containing EtOH-formulation which could reach deeper lung regions, but consecutively increasing the potential for exhalation in vivo. This could be an explanation for higher systemic availability, since ibuprofen as a small, particularly hydrophobic molecule is absorbed within seconds after inhalation and probably more rapidly from alveoli, if deposited there (Patton et al., 2004). Surprisingly, AUC and ibuprofen residues in lung tissue differed between the SFA/EtOH-groups. Although the MMAD of F6H8/EtOH in vitro was small enough for deep lung deposition (<2 μm), the drug release behavior differed from that of the F4H5/EtOH formulation. We initially assumed that ibuprofen was probably exhaled and was not deposited due to the lower vapour pressure of F6H8, consecutive less evaporation and a high fraction of droplets smaller than 1 μm (>45%). Surprisingly though, F6H8/EtOH was found to have the highest deposition of ibuprofen in lung tissue after 6 h. This finding may indicate a local deposition of ibuprofen even in dependent lung regions. Hence we conclude that the combination of F6H8 with co-solvent EtOH may lead to a slower drug release of the test drug with consecutive deposition in the lung tissue. Therefore, the systemic availability of ibuprofen and the drug deposition differences in these two groups cannot be explained by evaporation alone and has to be elucidated in further studies. At present, we conclude that the formulation containing F6H8 and EtOH leads to a "trapping" of the test drug and a more local drug release.

4.4. Limitations

Ibuprofen in water is only poorly soluble in water due to its hydrophobicity. The lower ibuprofen concentration in the control (H2O) formulation could interfere with the comparison with the higher concentrated SFA/EtOH formulations. Nonetheless applied ibuprofen dose (6 mg/kg bw) in the animal study was equal.

4.5. Potential future applications

F6H8 in combination with EtOH showed a long-lasting deposition of the test drug in dependent basal lung regions. This could make it attractive for the deposition of anti-inflammatory or antibiotic drugs after inhalative application in the ventilated critically ill patient. Inhalative therapies were developed not only for direct drug delivery into the diseased lung, but also for non-invasive systemic drug administration. Drug inhalation enables rapid deposition in the lungs and may induce fewer side effects than administration by other routes (Bailey and Berkland, 2009). As seen in all SFA and SFA/EtOH formulations Cmax could be reached within minutes. Accordingly for ibuprofen, this could be used to treat acute symptoms, such as pain. Furthermore, in contrast to oral delivery, where the drug is metabolized and altered by first pass effect in the liver, the lungs have only a small fraction of the drug-metabolizing activity of the gut and liver (Keith et al., 1987; Tronde et al., 2003).

4.6. Conclusion

The tested SFAs using ethanol as co-solvent are suitable for nebulisation and pulmonary drug delivery. Especially the F4H5/EtOH formulation might be used when rapid systemic availability is required. The F6H8/EtOH formulation showed an intrapulmonary deposition of the test drug. Further studies must clarify if this might be used for intrapulmonary drug targeting.

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