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Effects of perfluorohexane vapor in the treatment of experimental lung injury☆

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ABSTRACT

Rationale: We investigated the effects of vaporized perfluorohexane (PFH) on pulmonary vascular tone, pulmonary vascular resistance and peak inspiratory pressure as well as lipid mediator formation in the treatment of calcium ionophore induced lung injury in a model of the isolated perfused and ventilated rabbit lungs.
Methods: Lung injury was induced in isolated perfused and ventilated rabbit lungs by calcium ionophore A23187. Lungs were treated with either 4.5 vol.% (4.5 vol.% PFH; $n = 6$) or 18 vol.% (18 vol.% PFH; $n = 6$) PFH. Six lungs remained untreated (Control). In addition 5 lungs (PFH-sham) remained uninjured receiving 18 vol.% PFH only. Mean pulmonary artery pressure (mPAP), peak inspiratory pressure (P_{max}), and lung weight (weight) were monitored for 120 min. Experiments were terminated before when the increase in lung weight exceeded 40 g. Perfusate samples were taken at regular intervals for analysis of TXB_2, 6-keto-PGF_1 and LTB_4.
Results: Controls reached the study end point significantly earlier than both PFH groups. Significant differences were found for a weight gain of 10 g and 20 g between the control and the 4.5 vol.% PFH and the 18 vol.% PFH. Differences in mPAP were more pronounced in the 4.5 vol.% PFH. However increases in P_{max} were more marked in 4.5 vol.% PFH. TXA_2-, PGI_2-, and LTB_4-levels were significantly lower in PFH groups. Uninjured lungs remained unaffected by the presence of 18 vol.% PFH.
Conclusion: Inflammatory lung injury was attenuated by the treatment with 4.5 vol.% PFH and 18 vol.% PFH vapor in the isolated perfused rabbit lung. Therapeutic effects were more pronounced with a concentration of 4.5 vol.% PFH.

1. Introduction

Liquid ventilation with Perfluorocarbons (PFC) have been widely used for the treatment of experimental and clinical acute respiratory distress syndrome (ARDS) [1–6]. Despite promising results from early experimental and clinical trials [1–4] liquid ventilation failed in a recent phase III clinical trial [5]. Therefore, less invasive modes of PFC application have been developed whereby PFC was either vaporized [7] or aerosolized [8,9]. The inhalation of therapeutic agent for the treatment of ARDS is a well-established mode of application. Surfactant, prostacyclin and nitrous oxide have been successfully applied and were of therapeutic benefit in small single center studies [10,11]. In larger randomized trials these effects were less persuasive [12]. Several mechanisms of action have been suggested to contribute to the beneficial effects of inhaled PFC in the treatment of ARDS such as improved oxygen delivery and reduction of pulmonary surface tension [7,8,13]. In vivo studies with inhaled PFC documented a reduction in alveolar edema, vascular leakage, and inflammatory response [14–16]. In vitro studies using liquid PFC indicated further anti-inflammatory effects in alveolar macrophages [17] with a decrease in reactive oxygen species [18] and suggested

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that PFC may protect lung epithelial cells from neutrophil-mediated injury [19]. Due to their state of aggregation, inhaled PFCs may reduce the risks and adverse effects seen with total and partial liquid ventilation while exerting the beneficial effects of PFCs.

The aim of this study was to examine possible therapeutic effects of perfluorohexane (PFH) vapor and their dose-dependency on pulmonary function and lipid mediators in the isolated perfused and ventilated rabbit lung.

2. Material and methods

2.1. Animal preparation

The study protocol was approved by the Institutional Animal Care Committee and the Government of the State of Saxony, Germany and conformed to the National Institutes of Health guidelines for animal use.

Female rabbits ($n = 23$) weighing 2.8 ± 0.5 kg were anesthetized using ketamine, 50 mg/kg (CuraMED, Karlsruhe, Germany) and xylazine hydrochloride, 4 mg/kg (Bayer, Leverkusen, Germany) via the auricular vein. Subsequently all animals received 1000 U/kg heparin (Liquemin, Hoffman-La Roche, Grenzach-Wyhlen, Germany) intravenously. Following a tracheostomy, animals were ventilated with room air using a Servo 900C ventilator (Siemens-Elema, Solna, Sweden) (tidal volume: 8 ml/kg; respiratory frequency: 25 per min; positive end-expiratory pressure: 3 cm H_2O; inspiratory to expiratory ratio: 1:2).

After opening the thorax via a median sternotomy, heart and lungs were carefully prepared. Subsequently a perfusion catheter was placed in the pulmonary artery. The trachea, the lungs and the heart were removed en bloc from the thoracic cavity and the heart separated from the lungs. The trachea-lung organ bloc was than hung on a weight transducer (Hottinger Baldwin, Meßtechnik, Darmstadt, Germany) in a temperature controlled (37 °C) and humidified chamber.

Following the cannulation of the pulmonary artery the lungs were ventilated with 4% CO_2 in air while the ventilator setting remained unchanged. Initially lungs were perfused in an open circuit to remove remaining blood cells and cellular debris from the vascular bed with a Krebs-Henseleit solution (KHS) using low flow rates. After exchanging the perfusion fluid via two separate perfusion circuits at 10 and 20 min after the beginning of extracorporal circulation lungs were perfused in a closed recirculation system containing 200 ml KHS. The temperature of the perfusate was maintained at 37 °C and the pH between 7.35 and 7.45. Flow rates were successively increased to 150 ml/min (calibrated roller pump, Masterflex L/S, Cole–Parmer, Mfg. Barnant, Barrington, IL). After a steady state period of 30 min only lungs having a homogeneous perfusion, a constant airway pressure and a stable lung weight were included in this study.

2.2. Vaporization of perfluorohexane (PFH)

All experiments were performed using PFH ($CF_3(CF_2)_4CF_3$) (ABCR, Karlsruhe, Germany), whose physical and chemical characteristics have been described before [8,9]. PFH was vaporized using a modified vaporizer (Isoflurane Vaporizers 952, Siemens-Elema, Solna, Sweden) for Servo 900C ventilators. Concentrations of PFH were measured continuously by infrared spectroscopy (Iria, Dräger, Lübeck, Germany) and adjusted if necessary. The concentrations of 4.5 vol.% and 18 vol.% PFH applied in this study present the minimum and maximum concentration that can be delivered using this experimental set-up.

2.3. Experimental protocol

After an initial stabilization period of 30 min, 23 lung preparations were randomly assigned to two groups:

2.3.1. Uninjured lungs

- PFH-sham group (PFH-sham): Uninjured lungs ($n = 5$) received 18 vol.% PFH for a study period of 60 min and were followed up for another 60 min. Perfusate samples were taken before the beginning (t_{base}), and at 5 (t_5), 15 (t_{15}), 30 (t_{30}), 60 (t_{60}), 90 (t_{90}), and 120 (t_{120}) minutes after the beginning of PFH application.

2.3.2. Injured lungs

- PFH treatment group (PFH-Tx): Lungs ($n = 12$) were injured by adding calcium ionophore A23187 (Sigma, Deisenhofen, Germany) with a concentration of 1 μM to the perfusion fluid. After an increase of the mean pulmonary artery pressure of 25% from baseline (t_{injury}), lungs were treated with either 4.5 vol.% PFH (4.5 vol.% PFH-Tx; $n = 6$) or 18 vol.% PFH (18 vol.% PFH-Tx; $n = 6$) for 60 min followed by a 60 min observation period.
- Control group (Control): Lungs ($n = 6$) were equally injured by adding calcium ionophore A23187 at a concentration of 1 μM to the perfusion fluid but remained untreated. After an increase of the mean pulmonary artery pressure of 25% from baseline (t_{injury}), lungs were followed up for 120 min.

2.3.3. Interruption criteria

Experiments were terminated before the end of follow up period (t_{120}) when the increase in lung weight exceeded 40 g as ventilation was than almost impossible due to pulmonary edema.

2.3.4. Functional measurements

Mean pulmonary artery pressure (mPAP), lung weight (weight), and peak inspiratory pressure (P_{max}) were measured and recorded in 1 min intervals using a component monitoring system (CMS, Philipps, Böblingen, Germany). MPAP was assessed using an Ohmeda pressure transducer (GE Healthcare, Freiburg, Germany). Because of the constant perfusion flow, alterations of perfusion pressure directly reflected changes of pulmonary vascular resistance. P_{max} was measured by an integrated piezoresistive transducer within the Servo 900C ventilator. A complete set of measurements including perfusate samples was taken before induction of lung injury (t_{base}), at lung injury (t_{injury}) as well as 15 (t_{15}), 30 (t_{30}), 60 (t_{60}), 90 (t_{90}) and 120 (t_{120}) minutes after t_{injury}.

2.3.5. Measurement of lipid mediators

Perfusate samples were taken from the catheter that collects the effluent from the pulmonary veins to determine thromboxane A_2 (TXA_2), prostacyclin (PGI_2) and leukotriene B_4 (LTB_4) formation. TXA_2 and PGI_2 were measured by analyzing the concentration of their stable metabolites thromboxane B_2 (TXB_2) and 6-keto-prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) using the ELISA technique (Assay Designs, Ann Arbor, MI). Samples were drawn into 2 ml syringes containing 10 μg diclofenac (ASTA Medica AWD, Frankfurt, Germany) to stop the formation of TXA_2 and PGI_2. They were immediately centrifuged at 14 000 rpm and the supernatant subsequently frozen for later analysis. In addition LTB_4 concentrations were analyzed using the RIA technique which was performed according to the supplier's instructions (Amersham, Little Chalfont, England).