Vaporized Perfluorohexane Attenuates Ventilator-induced Lung Injury in Isolated, Perfused Rabbit Lungs

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Background: The authors tested the hypothesis that administration of vaporized perfluorohexane may attenuate ventilator-induced lung injury.

Methods: In isolated, perfused rabbit lungs, airway pressure–versus–time curves were recorded. At baseline, peak inspiratory pressure and positive end-expiratory pressure of mechanically ventilated lungs were set to obtain straight pressure–versus–time curves in both the lower and upper ranges, which are associated with less collapse and overdistension, respectively. After that, peak inspiratory pressure and positive end-expiratory pressure were set at 30 cm H2O and 0, respectively, and animals were randomly assigned to one of two groups: (1) simultaneous administration of 14% perfluorohexane vapor in room air (n = 7) and (2) control group—ventilation with room air (n = 7). After 20 min of cycling collapse and overdistension, tidal volume and positive end-expiratory pressure were set back to baseline levels, administration of perfluorohexane in the therapy group was stopped, and mechanical ventilation was continued for up to 60 min. Lung weight, mean pulmonary arterial pressure, and release of thromboxane B2 in the perfusate were measured. In addition, the distribution of pulmonary perfusate flow was assessed by using fluorescent-labeled microspheres.

Results: Significantly higher peak inspiratory values developed in control lungs than in lungs treated with perfluorohexane. In addition, upper ranges of pressure–versus–time curves were closer to straight lines in the perfluorohexane group. Lung weight, mean pulmonary arterial pressure, and release of thromboxane B2 were significantly higher in controls than in perfluorohexane-treated lungs. Also, redistribution of pulmonary perfusate flow from caudal to cranial zones was less important in the treatment group.

Conclusions: The authors conclude that the administration of perfluorohexane vapor attenuates the development of ventilator-induced lung injury in isolated, perfused rabbit lungs.

MECHANICAL ventilation may be life-saving in the setting of acute pulmonary failure by reestablishing an adequate gas exchange, but it also has the potential to exacerbate lung injury.1 If cyclic collapse–reopening of alveoli, as well as overdistension, are not avoided during mechanical ventilation, lung injury may be worsened or even initiated in previous nondiseased lungs, as demonstrated in experimental studies.2–4 Accordingly, the use of protective ventilation strategies aimed at minimizing the stress on lung parenchyma has been shown to attenuate lung injury and improve the outcome of patients with the acute respiratory distress syndrome (ARDS).5 However, because of the nonhomogenous distribution of injury, certain areas of the lungs of patients with ARDS may undergo cyclic collapse–reopening at any given airway pressure while other areas are being simultaneously overdistended.6 This observation suggests that mechanical stress may also occur with protective ventilation. Therefore, the combination of protective ventilatory strategies with pharmacologic therapies that could further attenuate the impact of ventilation on lung tissues seems reasonable.

The instillation of perfluorocarbons into injured lungs has been demonstrated to improve respiratory function in different experimental studies.7–11 Unfortunately, however, such a strategy has not been able to improve outcome when used in combination with a less aggressive ventilation in a human multicenter trial.12 Alternative application forms of perfluorocarbons, e.g., aerosol and vapor, are also capable of treating experimentally induced lung injury.13–16 The administration of perfluorocarbon vapor, more specifically of perfluorohexane, is particularly interesting because of the ease of its application and because it does not lead to the formation of a liquid phase within the lungs, interfering only minimally with the respiratory pattern. Because perfluorohexane is administered as a vapor, this substance is distributed directly to ventilated regions that may be damaged by mechanical ventilation. In addition, potential adverse effects of filling the lungs with a liquid perfluorocarbon, e.g., transitory hypoxia and impairment of hemodynamics, barotrauma, and formation of liquothoraces, can be avoided in this new approach. Although those characteristics make perfluorohexane suitable for use in combination with protective ventilation, it is currently not known whether this approach is able to protect from ventilator-induced lung injury (VILI). In the current study, we aimed to determine whether the application of perfluorohexane vapor is able to attenuate the development of VILI.

Materials and Methods

This study was conducted with approval of the Committee for Experimental Research of the Carl Gustav Carus University Hospital, Technical University Dresden, Germany, and the protocol was in accordance to the Guidelines for Animal Use of the National Institutes of Health.
Preparation of Animals

The preparation of the isolated, perfused rabbit lung model has been described in detail by our group. Briefly, female rabbits (Oryctolagus cuniculus) weighing 1.6–2.6 kg were anesthetized with 50 mg/kg ketamine (CuraMED, Karlsruhe, Germany) and 4 mg/kg xylazine hydrochloride (Bayer, Leverkusen, Germany) after cannulation of the auricular vein. Heparin, 1,000 U/kg (Liquemin; Hoffmann-La Roche, Grenzach-Wyhlen, Germany), was administered intravenously for anticoagulation. After skin infiltration with 8 ml lidocaine hydrochloride, 1% (Jenapharm, Jena, Germany), a tracheotomy was performed, and the trachea was cannulated using a 10-cm-long catheter with a diameter of 0.4 cm (endotracheal tube) (B. Braun, Melsungen, Germany). Animals' lungs were ventilated with room air using the Small Animal Ventilator KTR-4 (Hugo Sachs Elektronik GmbH, March, Germany). The initial ventilator settings were tidal volume (Vₜ) of 8 ml/kg, with a constant respiratory flow adjusted to obtain the desired Vₜ and compensate for the minimal losses of the respiratory circuit (typically approximately 0.6 l/min); respiratory frequency of 30 breaths/min; positive end-expiratory pressure (PEEP) of 1 cm H₂O; and inspiratory:expiratory ratio of 1:1. PEEP was set using an external water column connected to the expiratory port of the ventilator.

After a median sternotomy, the pulmonary artery was cannulated, and the heart was opened to permit the exsanguination of the lungs with a Krebs-Henseleit hydroxyethyl starch buffer solution (perfusate), which was pumped with a roller pump at 50 ml/min (Masterflex L/S; Cole-Parmer, Mfg. Barnant, Barrington, IL). The lungs and trachea were carefully dissected, removed en bloc, and suspended from a weight transducer (Hottinger Baldwin Meßtechnik, Darmstadt, Germany) in a temperature-controlled (37°C), double-walled chamber. The lung per fusate, which circulated through the lungs and trachea were carefully dissected, removed en bloc, and suspended from a weight transducer (Hottinger Baldwin Meßtechnik, Darmstadt, Germany) in a temperature-controlled (37°C), double-walled chamber. The lung perfusate flow rate was increased to 100 ml/min, and the whole volume was exchanged two times before the beginning of the measurements.

Mean Pulmonary Artery Pressure and Lung Weight

Mean pulmonary artery pressure (MPAP) was monitored continuously by means of a differential pressure difference transducer and using the CMS Monitor (Agilent Technologies, Böblingen, Germany). The MPAP was zero-referenced at the hilus height. The weight transducer was also connected to the CMS Monitor, and both lung weight and MPAP were recorded each minute by means of a microcomputer.

Administration of Perfluorohexane

Perfluorohexane (C₆F₁₄) (ABCR, Karlsruhe, Germany) with a purity of 95% was used in this study. This perfluorocarbon has similar physicochemical properties with common volatile anesthetics (e.g., vapor pressure at 20°C of 177 mmHg, boiling point of 57°C) and is therefore suitable for administration with commercial vaporizers. In this study, we used two bypass vaporizers of type 19 n (Drägerwerk AG, Lübeck, Germany), which were connected in series in the inspiratory limb of the mechanical ventilator to avoid the need for refilling during the treatment period. The dosage cones of the vaporizers were modified by the manufacturer to allow administration of perfluorohexane vapor concentrations as high as 14% at room temperature. Perfluorohexane concentrations were measured by infrared spectroscopy using a sidestream (200 ml/min) gas measurement device (IRIA®, Drägerwerk AG). This device was adapted and calibrated by the manufacturer using the IR-Spektrometer (Bruker, Leipzig, Germany) as reference.

Respiratory Mechanics

The transpulmonary pressure (Pₜ) was measured as the airway pressure at the Y-Piece of the mechanical ventilator using a differential pressure transducer referenced to the atmosphere (PasCal; Hoffrichter GmbH, Schwerin, Germany). The Pₜ signal was digitized at 200 Hz by an analog–digital board (DAQ-Pad 1200; National Instruments, Austin, TX) with 10 bits and acquired by a laptop using a special routine developed for LabView® (National Instruments).

Records of pressure-versus-time curves were obtained during constant flow inflation over three consecutive respiratory cycles. The signals were processed off-line to determine the degree of lung collapse and overdistension according to a method described in detail in our recently work. Briefly, equations 1 and 2 were fitted to the first lower third and the upper two thirds of the dynamic pressure-versus-time curve, respectively:

\[
P_{\text{lower}} = a_{\text{lower}} \cdot b_{\text{lower}} + c_{\text{lower}}
\]

\[
P_{\text{upper}} = a_{\text{upper}} \cdot b_{\text{upper}} + c_{\text{upper}}
\]

where coefficients \(a_{\text{lower}}\) and \(a_{\text{upper}}\) are constants that represent the slope of the pressure-versus-time relation and coefficients \(c_{\text{lower}}\) and \(c_{\text{upper}}\) represent the pressures at the beginning of the respective curves. The coefficients \(b_{\text{lower}}\) and \(b_{\text{upper}}\) are dimensionless constants that describe the concavity of the lower and upper portions of the pressure-versus-time curve, respectively. For values greater than 1, the dynamic pressure-versus-time curve has an upward concavity, indicating that the compliance decreases with time, as, for example, when...
the lungs are being overdistended. For values less than 1, the lower portion of the dynamic pressure-versus-time curve has a downward concavity, indicating that the compliance increases with time, as, for example, when the lungs are being inflated from collapse. For \( b_{\text{lower}} \) and \( b_{\text{upper}} \), values equal to the unity dynamic pressure-versus-time curves are straight lines, indicating a constant lung compliance without collapse or overdistension. Because the \( P_I \) rise has a characteristic spike when the inspiratory flow starts, flow curves were not necessary to identify the beginning of inspiration. To ensure that on-and off-flow transients generated by the mechanical ventilator did not skew the results, the first and last 50 ms of the inspiratory cycle were excluded from the analysis. These values have been used in our recent publication and are virtually the same as those proposed by Ranieri et al. in a similar procedure.

Measurement of Thromboxane B₂

Perfusate samples were taken with 2-ml syringes containing 13.6 μg dicyclofenac (Rewodina; ASTA Medica AWD, Frankfurt, Germany) and immediately centrifuged at 14,000 rotations/min for 10 min. Using a calibrated pipette, 1,000 μl of the samples was drawn and frozen at −20°C. Afterward, samples were thawed, and thromboxane B₂ concentrations were measured in a blinded fashion using enzyme-linked immunosorbent assay (Institute of Biochemistry, University Clinic Carl Gustav Carus, Dresden, Germany).

Experimental Protocol

Initially, PEEP was increased until a straight line was achieved in the lower part of the dynamic press-versus-time curve \( (b_{\text{lower}} \approx 1) \). After that, the lungs were gently recruited with sustained inflation at 30 cm H₂O for 30 s, and \( V_T \) was set at 6–8 ml/kg to obtain a straight line also in the upper portion of the dynamic pressure-versus-time curve \( (b_{\text{upper}} \approx 1) \). After that, the lungs were randomized to one of two groups: the therapy group or the control group.

Therapy Group \( (n = 7) \). In the therapy group, the inspiratory flow rate, and consequently \( V_T \), was increased up to a peak inspiratory pressure (PIP) of 30 cm H₂O to produce lung overdistension. Also, PEEP was set at zero, and the respiratory frequency was reduced to 20 breaths/min to allow lung collapse at end expiration. Simultaneously, the control dials of the vaporizers were switched to the position equivalent to maximal concentration. Using the IRIA® device, we could assure that no perfluorohexane was administered, i.e., that perfluorohexane was equal to 0%. As in the therapy group, the lungs were ventilated with these settings for 20 min, when the control dials were switched off and the protective ventilation was resumed and kept for 60 min.

After the injurious ventilation period, PEEP was set at the same value as before challenge (table 1).

Sequence of Measurements

Measurements of respiratory mechanics were performed before randomization (baseline) and at 0, 30, and 60 min after the challenge with ventilation, with high \( V_T \) values and zero PEEP. Perfusate samples were drawn at baseline and at 0 and 60 min.

Determination of Pulmonary Capillary Perfusate Flow Distribution

In four animals of the control group and three animals of the therapy group, distributions of pulmonary capillary perfusate flow were determined at baseline and 60 min after the ventilatory challenge (time 60) by means of the color-labeled microspheres method, which has been described in detail elsewhere. Briefly, fluorescent polystyrene microspheres (yellow-green, red, and crimson) of 15-μm diameter (Molecular Probes, Eugene, OR) were used to determine regional perfusate flow in the isolated lungs. Immediately before injection, the microspheres were vortexed and then sonicated for 90 s. The number of microspheres per injection was approximately \( 1.0 \times 10^5 \). The injection of the microspheres was performed over 60 s using a side port close to the tip of the catheter placed in the pulmonary artery. The fluorescent colors were randomized in every experiment.

After completion of the study protocol, the catheters were removed, and the lungs were inflated to 20 cmH₂O and dried with air for 2 days. Then, the lungs were coated with a one-component polyurethane foam (BTI Befestigungstechnik GmbH & Co. KG, Ingelheim, Germany), suspended vertically in a square box, and embedded in rapidly setting urethane foam (polyol and isocya-
The foam block was cut into uniformly sized cubes of 1 cm³ in volume. Foam adhering to the lung pieces was removed. Each cube was weighed and assigned a three-dimensional coordinate. Samples with airways occupying more than 50% of the cube’s volume were discarded. They were then individually soaked for 2 days in 2 ml 2-ethoxyethyl acetate (Aldrich Chemical Co., Milwau-kee, WI) to retrieve the fluorescent dye. The fluorescence was read in a luminescence spectrophotometer (Perkin-Elmer LS-50B; Beaconsfield, Buckinghamshire, United Kingdom) fitted with a flow cell and a standard photomultiplier tube. The weight-normalized relative perfusate flow at each time point was calculated for each lung piece according to the following equation:

\[
\frac{Q_{\text{rel},i}}{H_{11005}x_i/H_{20849}/H_{20858}x_i/H_{20850}/n} (3)
\]

where \(Q_{\text{rel},i}\) is the weight-normalized relative perfusate flow of the piece \(i\), \(x_i\) is the fluorescence divided by the weight of the piece, and \(n\) is the number of pieces of the lung. The mean normalized relative flow was therefore 1.0.

Statistical Analysis
All results are expressed as mean ± SD. Comparison between groups was performed using two-way analysis of variance with two-way entry (group, time; general linear model). Multiple comparisons were performed with the Student–Newman–Keuls correction. A linear regression analysis was performed to assess the relation between the relative flow and the distance from the caudal lung zone (distribution along the caudal-to-cranial axis). Comparison of the slopes of regression lines from pulmonary capillary perfusate flow was performed by means of unpaired \(t\) tests. A \(P\) value less than 0.05 was considered to be statistically significant in all tests performed. Data were analyzed using the commercial software SPSS for Windows, version 11.0 (SPSS, Chicago, IL).

Results
There were no significant differences between groups at baseline with respect to animal weight, \(V_T\), and PEEP settings (table 1).

Respiratory Mechanics
Figure 1 shows typical pressure-versus-time curves obtained in lungs from each group at the different time points. At baseline and time 0, the lower and upper portions of the curve were straight lines in both groups. At times 30 and 60, the pressure-versus-time curves of the lungs from the animal of the control group show a slight downward concavity in the lower portion and an upward concavity in the upper portion (fig. 1, top), which were associated with inflation from collapse and overdistension, respectively. Conversely, the pressure-versus-time curves remained straight lines in both lower and upper portions throughout the experiment in the group treated with 14% perfluorohexane vapor, although angular coefficients change and curves became steeper (fig. 1, bottom). As can also be noticed in figure 1, the maximal pressure achieved during inspiration increased more importantly in the lungs from control than in the lungs from therapy animals.

The deviation of the coefficient \(b_{\text{lower}}\) from the unit is presented in figure 2A. For both groups, deviation values were close to zero at baseline but decreased significantly immediately after the ventilatory challenge (time 0, \(P < 0.05\) and 30 min thereafter (time 30, \(P < 0.05\)). However, differences between groups were not statistically significant.
The deviation of the coefficient $b_{\text{upper}}$ from unit is presented in figure 2B. The pressure-versus-time relation at the upper portion of the curve was almost linear at baseline and time 0 in both groups, leading to deviation values not significantly different from zero. As a result of the ventilatory challenge, the deviation of the coefficient $b_{\text{upper}}$ from unit increased significantly at time 30 ($P < 0.05$) and time 60 ($P < 0.01$) as compared with baseline. However, the mean value of $b_{\text{upper}}$ was higher in the control group than in the therapy group at time 60 ($P < 0.01$).

The PIP levels achieved in both groups were comparable at baseline and time 0 but increased over the observation period, achieving statistical significance at time 60 ($P < 0.01$). Nevertheless, PIP values were lower in the therapy group than in the control group by the end of the observation period ($P < 0.01$; fig. 3A).

**Mean Pulmonary Artery Pressure**

There was no statistically significant difference in mean MPAP at baseline between groups (fig. 3B), but values increased significantly at time 60 ($P < 0.01$). In the control group, MPAP increased more importantly than in the therapy group at 60 min ($P < 0.01$), achieving levels as high as 90 mmHg.

**Lung Weight**

Values of lung weight were comparable between groups at baseline. Also, there were no statistically significant differences in lung weight between the control and therapy groups at time 0 and time 30 (fig. 3C). Thereafter, an increase in lung weight could be observed in almost every animal ($P < 0.01$), with higher levels being achieved at time 60 in the control group as compared with the therapy group ($P < 0.01$).

**Thromboxane B$_2$ Concentration**

The concentration of thromboxane B$_2$ in the perfusate was below the limit of 10 pg/ml at baseline and comparable between groups at times 0 and 30 (fig. 3D). The thromboxane B$_2$ concentration increased during the observation period ($P < 0.05$) and was significantly higher in the control than in the therapy group at time 60 ($P < 0.05$).

**Distribution of Pulmonary Capillary Perfusate Flow**

Figure 4 shows typical distributions of pulmonary capillary perfusate flow along the caudal-to-cranial axis. In both control and therapy lungs, a tendency was observed that pulmonary capillary perfusate flow was stronger in caudal than cranial lung zones at baseline, following the gravity gradient, as suggested by negative slopes of the regression lines at baseline. In the animal of the control group, there was an important shift of pulmonary capillary perfusate flow from caudal to cranial zones at time 60, as evidenced by the positive angular coefficient of the regression line. For the animals of the therapy group, reversal of distribution along the caudal-to-cranial axis was less important at time 60.

Slopes of regression lines were comparable for animals of the control and therapy groups at baseline along the caudal-to-cranial axis (table 2). However, at time 60, slopes became significantly higher in animals of the
control group as compared with animals of the therapy group ($P < 0.05$).

**Discussion**

Mechanical ventilation has the potential to perpetuate, aggravate, or even initiate lung injury if the mechanical stress of lung parenchyma exceeds a certain limit, which may vary widely according to the underlying physiologic and morphologic alterations of the lungs.\(^2\) \(^-\) \(^4\) Soon after ARDS was first described, Mead *et al.*\(^2\)\(^1\) recognized that the distribution of mechanical stresses during mechanical ventilation in a nonhomogenous diseased lung may lead to an amplification of forces in regions surrounding atelectatic areas. Those forces, which were associated with PIP values as low as 30 cm H\(_2\)O, may lead to shear stress of lung tissues due to cyclic opening and collapse of alveoli. Moreover, \(V_T\) values of 12 ml/kg have been shown to cause alveoli overdistension in patients with ARDS, contributing to further mechanical stress.\(^2\) \(^2\) Therefore, strategies aimed at protecting the lungs from overdistension and collapse have become part of the standard of care in patients with ARDS. However, even when \(V_T\) values and PEEP are set according to a protective strategy, the mechanical stress in certain lung regions may achieve levels that are injurious for the pulmonary tissue. This phenomenon can be explained by nonhomogenous distribution of air and fluid in the lungs of patients with ARDS,\(^6\) and therefore, certain areas may be more prone to injury induced by ventilation than others. Theoretically, the use of adjunctive therapies combined to protective ventilation could be beneficial in such a context.

Among the substances that could be used in combination with protective ventilatory strategies, perfluorocarbons are particularly interesting. Because of their high oxygen-carrying properties, perfluorocarbons have been extensively investigated as a means for maintaining pulmonary gas exchange. However, filling the lungs with a liquid phase may be associated with transitory oxygen...
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Fig. 4. Distribution of relative perfusate flow along the caudal-to-cranial axis in illustrative lungs of the control and therapy (14% perfluorohexane vapor in room air [perfluorohexane 14%]) groups. Values in the y-axis represent distance from caudal zone in the head-up positioned lung, whereas values in the y-axis represent the normalized (relative) perfusate flow. Straight lines represent linear regression lines. $R^2 =$ coefficient of determination.

desaturation and also late complications as, for example, liquothoraces.25 Moreover, the combination of partial liquid ventilation (PLV) with a protective ventilatory strategy has not been shown to improve outcome when compared with protective gas ventilation alone.12

The limitations associated with PLV led different groups to search for alternatives for the application of perfluorocarbons to the lungs. Kandler et al.13 demonstrated that FC 77 aerosol is able to improve pulmonary mechanics and gas exchange in an ARDS model. The accumulation of droplets of perfluorocarbon can build a liquid phase in the lungs, which may be responsible, in part, for the positive effects observed with this approach. Nevertheless, the amount of perfluorocarbon administrated as aerosol is much lower than the amount used during PLV, and a “volume effect” is less evident.15 Recently, Bleyl et al.15 and Hübler et al.24 demonstrated that the administration of perfluorohexane vapor is able to reduce lung injury induced by oleic acid in sheep. Theoretically, the formation of a liquid phase of perfluorohexane within the lungs, i.e., condensation, is not possible, because the temperature at which the perfluorocarbon was vaporized is the lower than the temperature of the lungs. Although we did not measure the amount of perfluorohexane retained in the lungs, the observation that lung weights were comparable after administration of the vapor seems to confirm this claim. Further studies with perfluorohexane have also demonstrated the ability of this substance to reduce the proinflammatory and procoagulatory activity of alveolar macrophages,25 but the mechanisms by which perfluorohexane vapor or other perfluorocarbon compounds and application forms protect against the development of lung injury are not yet completely understood.

This study adds new important insight to those previous works. Our results suggest that perfluorohexane vapor is able to reduce the effects of the mechanical stress of ventilation on the lung parenchyma, which could be in part responsible for the improvement in respiratory function observed in previous works.15,24 Other studies have also addressed the potential of perfluorocarbons to attenuate injury induced by mechanical ventilation. Vasquez de Anda et al.26 have shown that PLV is able to improve pulmonary function in a rodent model of VILI after the ventilatory challenge has occurred. Accordingly, Ricard et al.27 have demonstrated that PLV contributes to minimize VILI by redistributing ventilation during hyperinflation in rats. Although our results are in accord with the claim that administration of perfluorocarbons may reduce the impact of ventilation on the lung parenchyma, comparison with previous reports is limited by the fact that we used only the vapor phase of a perfluorocarbon. In our study, dynamic compliance was better preserved in lungs treated with perfluorohexane than in controls after a ventilatory challenge, as evidenced by lower PIP values. Although the deviation of the coefficient $b,lower$ from unit occurred in both groups, values of the coefficient $b,upper$ were significantly higher in the control group. These observations suggest that overdistension, but not collapse, occurred at a lesser degree after treatment with perfluorohexane. The mechanical stress of ventilation may lead to disruption of the alveolocapillary membrane resulting in pulmonary edema,28 with an increase in lung weight. In our study, perfluorohexane-treated lungs showed less increase in lung weight, suggesting that the alveolocapillary membrane was better preserved. According to Obraztsov et al.,29 erythrocytes exposed to perfluorooctyl bromide become resistant to hemolysis in hypotonic solution. Those authors29 speculated that partition of perfluorocarbon into the lipid component of the erythrocyte cellular membrane changed their properties. Also, it has been suggested that perfluorocarbons may act as a physical barrier to alveolar flooding, redistributing ventilation, and edema during mechanical ventilation.27 Because a liquid phase of perfluorohexane was

Table 2. Slopes of the Linear Regression Lines of Perfusate Flow Distributions along the Caudal-to-cranial Axis

<table>
<thead>
<tr>
<th>Animal</th>
<th>Baseline</th>
<th>Time 60</th>
<th>Baseline</th>
<th>Time 60</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-0.1605</td>
<td>0.2978</td>
<td>-0.1486</td>
<td>0.0267</td>
</tr>
<tr>
<td>2</td>
<td>-0.4387</td>
<td>0.1648</td>
<td>-0.3818</td>
<td>0.0263</td>
</tr>
<tr>
<td>3</td>
<td>-0.0936</td>
<td>0.5851</td>
<td>-0.1173</td>
<td>-0.0642</td>
</tr>
<tr>
<td>4</td>
<td>-0.2132</td>
<td>0.2604</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>-0.2265</td>
<td>0.3270</td>
<td>-0.2159</td>
<td>-0.0037</td>
</tr>
</tbody>
</table>

* $P < 0.05$ vs. therapy group.
Control = room air; 14% perfluorohexane = therapy with 14% perfluorohexane vapor in room air.
not present in the lungs treated in our study, partition of perfluorohexane into cellular membranes with subsequent increased resistance to stretching is the most probable explanation for our results. Also, an antiinflammatory mechanism may have had a role, but those claims remain speculative.

The development of pulmonary edema during VILI may result not only from changes of microvascular permeability but also from increased hydrostatic pressure. Nevertheless, Carlton et al. have demonstrated that high-airway-pressure ventilation does not lead to considerable increase of mean transmural microvascular pressures in lambs ventilated with closed chest, suggesting that increase of hydrostatic pressure is not an important factor in the genesis of VILI in that model. Although an increase of transmural microvascular pressure seems to be more significant in experiments involving open-chest, high-airway-pressure ventilation, the role of the hydrostatic component is also considered to be secondary even in such conditions. Therefore, the most probable explanation for the increase in MPAP observed in our study lies on the triggering of mediators with vasoconstrictory properties. The disruption of cell membranes may be associated with activation of phospholipase A2 and fatty acid mobilization. Higher amounts of substrate for the cyclooxygenase and 5-lipoxygenase enzymes result in increased production of eicosanoids that may cause vasoconstriction or increase of capillary permeability or both. The activation of the cyclooxygenase pathway as a mechanism for increased MPAP values is supported by our data, which showed higher thromboxane B2 concentrations in control lungs than in lungs treated with perfluorohexane. Thromboxane B2 is the stable metabolite of thromboxane A2, which is a potent vasoconstrictor. When hydrostatic pressure is increased in an environment of increased microvascular permeability, alveolar flooding may occur. In fact, we could visually observe that tracheal flooding developed in most lungs late in the course of VILI.

According to different authors, distribution of pulmonary capillary blood flow of head-up positioned isolated rabbit lungs ventilated with moderate VT values followed the gravity gradient, with caudal zones being stronger perfused than cranial ones. Our results are in accord with those reports. However, after the respiratory challenge, perfusate flow was redistributed from caudal to cranial zones. Although some degree of redistribution could be observed in all lungs studied, the shift of pulmonary perfusate flow was much more important in controls than in lungs treated with perfluorohexane vapor. This pattern of redistribution was also observed by Hübler et al. during administration of perfluorohexane in a surfactant depletion model of lung injury. Accordingly, Loer et al. reported a similar pattern during PLV and attributed this effect to an increase in hydrostatic pressure in caudal zones. Because a liquid perfluorocarbon phase was not present in our study, the hydrostatic pressure of alveolar flooding may have been responsible for the more significant shift in pulmonary perfusate flow in control lungs. Administration of perfluorohexane vapor per se seems not to influence the distribution of pulmonary capillary perfusate flow in isolated rabbit lungs.

Limitations of the Study

This study has several important limitations. The most important one is in regard to the fact that VILI induced in isolated rabbit lungs represents only an approximation of the in vivo situation. Therefore, it should be kept in mind that this model does not reproduce all features of the much more complex clinical scenario. We decided to use isolated rabbit lungs for two main reasons. First, influence of extrapulmonary organs with metabolism of inflammatory mediators released during VILI should not be present. Second, fluctuations of perfusion due to impaired hemodynamics or cardiopulmonary interactions or both, which may influence the course of VILI, would be minimized.

We must also acknowledge that the PIP values were set at 30 cm H2O during the ventilatory challenge, although such airway pressures are considered to be safe in patients with ARDS. However, the isolated and perfused rabbit lungs were situated in an environment with barometric pressure conditions, and simulation of the chest wall was not performed. Therefore, lung expansion was unopposed by external forces, and end-inspiratory lung volumes were much higher than in a closed chest. In fact, inspiratory flow rates, which are directly proportional to VT, had to be increased up to threefold to fourfold baseline values to achieve PIP values of 30 cm H2O. In addition, VILI may develop in small animals with pressures lower than those necessary to cause lung injury in large animals. Another possible limitation of our study regards the length of exposure to injurious ventilation. The relatively short period of 20 min may have contributed to a nonhomogeneous pattern of injury among animals, increasing also the variability of parameters measured. Because of this high degree of variability, the possibility of a β error cannot be ruled out.

Our study is also limited by the fact that the observation time after the respiratory challenge was fixed at 60 min. The reason for choosing that relatively short period is that a longer time would not have been possible because of dramatic alveolar flooding in lungs in which VILI developed. Because of that limitation, other mechanisms postulated to be involved in the genesis of VILI, which require longer times, as, for example, transduction of mechanical stress into gene expression and production of cytokines and other proinflammatory substances, probably did not have an important role in our study.

Because of all of these limitations, extrapolation of the values used in our study or conclusions derived from this...
model to the clinical scenario may not be appropriate. Clearly, in vivo studies are required before one can extend our findings to the clinical arena.

Finally, our study was observational in nature, and its design did not permit to identify the mechanisms by which perfluoroheptane vapor may exert a protective effect against VILI. This issue remains to be clarified.

In summary, administration of 14% perfluoroheptane vapor during a ventilatory challenge with high PIP values and zero PEEP is able to attenuate the subsequent deterioration of pulmonary mechanics, increase of lung weight, development of pulmonary hypertension, and release of thromboxane \( \beta_2 \) and also to minimize the redistribution of pulmonary capillary perfusate flow in isolated, perfused rabbit lungs. Further studies are needed to identify the mechanisms by which perfluoroheptane vapor may attenuate lung injury induced by mechanical stress and to confirm our findings in an in vivo model.

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