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Animal Studies on Lung Function

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PREFACE

Various mechanical ventilation techniques have been investigated, both experimentally and clinically, to improve outcome from acute respiratory failure. As an alternative means of respiratory support, perfluorocarbon (PFC) liquids have gained interest first in liquid breathing applications, and then have formed the basis for the liquid ventilation technique.

Combining the liquid and gas ventilation techniques, a new ventilatory support technique, namely partial liquid ventilation, is in progress as an investigational treatment modality in which gas tidal ventilation is superimposed on PFC-treated lungs.

This thesis consists of six articles focusing on this topic. One review and five articles describing original experimental work on partial liquid ventilation are presented. Partial liquid ventilation is investigated with respect to its effects on pulmonary gas exchange and respiratory system mechanics in an animal model of acute respiratory failure and in healthy animals.

OVERVIEW OF THE STUDY

Chapter 1 begins with a description of the total liquid ventilation technique. PFC liquids and their biomedical applications are briefly described. The related literature on liquid ventilation in healthy animals and in acute respiratory failure are separately outlined. The results of the first clinical application liquid ventilation are discussed. In the second part, the partial liquid ventilation technique is discussed with reference to some of the related literature.

In Chapter 2, the first study on partial liquid ventilation in animals with induced acute respiratory failure is presented. The dose-related effects of intratracheal PFC administration on pulmonary gas exchange, lung mechanics and hemodynamic stability at the short term are documented in comparison to a saline control group.

Chapter 3 presents a study which compares partial liquid ventilation with conventional mechanical ventilation (continuous positive pressure ventilation-CPPV). In this study, animals are treated with a PFC volume corresponding to functional residual lung capacity (18 ml/kg) during partial liquid ventilation. Conventional ventilator settings were applied during both partial liquid ventilation and CPPV for 3 h, and respiratory parameters and post-mortem lung histology are compared.

In Chapter 4, the effects of different doses of intratracheal PFC administration on respiratory parameters are tested in adult animals with acute respiratory failure during a 6-h observation period. Our explanations for the mechanism of action of partial liquid ventilation for improving gas exchange and lung mechanics are discussed. The dynamic pressure-volume measurement is documented in the lung-lavaged animals during

administration of PFC at incremental doses. Additionally, the data from healthy controls are presented in order to determine the effects of PFC itself, as well as the time course on the experimental preparation.

In Chapter 5, the effects of partial liquid ventilation on lung function in healthy adult animals are investigated. For this purpose, respiratory parameters are tested during 3 h of partial liquid ventilation to determine the short-term effects, and on the seventh day following partial liquid ventilation for the long-term effects. Comparison with control pre-treatment data is made at different PEEP levels for respiratory parameters.

Chapter 6 presents a study investigating the effects of partial liquid ventilation on healthy lung function, using a more sensitive technique. The measurement of pulmonary clearance of a radio-labelled tracer (^{99m}Tc-DTPA) is a more sensitive and detailed test for studying the functional integrity of the alveolo-capillary barrier (surfactant system). Clearance measurements are performed after a 3-h period of partial liquid ventilation and compared to the control data during conventional mechanical ventilation.

CHAPTER 1

PERFLUOROCARBONS AS AN ALTERNATIVE RESPIRATORY MEDIUM

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PERFLUOROCARBONS AS AN ALTERNATIVE RESPIRATORY MEDIUM

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INTRODUCTION

Acute respiratory failure (ARF) represents one of the major problems in intensive care units because of its complex pathogenesis and high rates of morbidity and mortality. Since the clinical problems are similar regardless of the etiology of the lung injury, the therapeutic goal in ARF is to support patients until alveolocapillary membrane integrity is reestablished. Therefore, the main critical factors in clinical management of ARF are supply of adequate arterial and tissue oxygenation and treatment of the primary disorder. Different ventilatory support techniques have been introduced in respiratory failure to maintain adequate gas exchange in the lung with the least possible barotrauma and circulatory impairment.

Mechanical ventilation with high inflation pressures is associated with an increased incidence of barotrauma. Based on extensive evidence accumulated from animal studies, showing that high tidal volumes and high peak inspiratory pressures can induce or even worsen acute lung injury [3-5], different ventilatory techniques have been investigated during the last 10 years. Hickling et al. demonstrated that mortality could be significantly

reduced in patients with severe respiratory distress syndrome (RDS) by limiting peak inspiratory pressure (reducing tidal volume) and disregarding hypercapnia [6]. Other investigators have shown improved oxygenation with decreased airway pressures using pressure-controlled inverse ratio ventilation [7,8]. High frequency jet ventilation has been proposed to achieve effective gas exchange using low tidal volumes at high respiratory rates [9,10]. Extracorporeal membrane oxygenation (ECMO) and apneic oxygenation with extracorporeal CO_2 elimination have been applied in severe cases as nonconventional ventilatory support techniques where other ventilatory forms have failed to improve gas exchange. Although ECMO has resulted in clinical failure [11], the latter appears to be a promising technique by reducing mortality rates in patients with RDS [12]. The reestablishment of the functional integrity of the damaged surfactant in RDS in adults by exogenous surfactant replacement has been limited to few case reports [13,14] because of nonavailabilty of surfactant in the required amounts and the high cost for adults.

Although more than two decades of clinical and labarotory research on RDS have passed, survival rates have remained virtually unchanged [2]. In clinical conditions, the diversity of the disorders associated with RDS has made it difficult to define the optimal ventilatory strategy. However, no controlled studies have demonstrated the superiority of one technique over other forms of ventilatory support. For these reasons, there has always been a search for ways to improve the clinical management of RDS.

As an alternative means of respiratory support, perfluorocarbon (PFC) liquids have been investigated extensively in liquid ventilation studies in animals, and this concept has led recently to the use of this new technique in preterm human neonates with severe

RDS in whom conventional therapies have failed [15]. Although the technique of liquid ventilation has evolved since it was introduced first by Clark and Gollan [16], the search for a simpler means of PFC application has given rise to a new method combining conventional gas ventilation and liquid ventilation, namely partial liquid ventilation [17-19]. In this new technique, PFC is applied intratracheally while mechanical ventilation at conventional ventilator settings is superimposed on partly PFC-filled lungs.

This review summarizes the literature on liquid ventilation and partial liquid ventilation, and describes the rationale behind partial liquid ventilation and, finally, the limitations and implications of partial liquid ventilation for future clinical application.

LIQUID VENTILATION

Perfluorocarbons

The most important physical properties of PFC liquids which make them suitable for ventilatory purposes are their remarkably low surface tension and extraordinary ability to dissolve oxygen (O_2) and carbon dioxide (CO_2) (Table 1).

Table 1. Physical properties of some PFC liquids.

	Perflubron	FC-77	RM-101
Density (g/ml)	1.92	1.75	1.77
Vapor pressure (mmHg at 37°C)	10.5	75	64
Surface tension (dynes/cm)	18	14	15
O_2 solubility (ml/100 ml)	53	56	52
CO_2 solubility (ml/100 ml)	210	198	160

PFC liquids are relatively simple organic compounds in which all hydrogen atoms have been replaced by fluorine; in addition, these chemicals also include compounds in which other halogen atoms such as bromine and iodine are present. These colorless, clear and odorless liquids are insoluble in aqueous media and they must first be emulsified before intravenous administration. Perfluorocarbons are very stable, chemically and biologically inert liquids due to the strong bond between carbon and fluorine atoms and can not be metabolized in biological systems. PFC liquids are primarily eliminated by expiration from the lungs and, possibly, to a very minor degree via transpiration through the skin [20]. When administered into the lungs, systemic absorption and distribution of PFC to the other tissues in small amounts have been demonstrated and measurements in expired gases have documented a rapid elimination of PFC through the lung [21,22]. The physico-chemical properties of PFC liquids are described in details elsewhere [23].

Biomedical Applications of Perfluorcarbons

As an oxygen carrier, PFC liquid is employed in various areas, either intravenously in emulsified form or intratracheally in neat (unemulsified) form. Since the first successful trial of Clark and Gollan on liquid breathing with PFC [16], liquid ventilation has been intensively investigated to support pulmonary gas exchange both in healthy and diseased lungs. PFC liquid breathing was administered also in other respiratory environment studies such as during deep diving and exposure to hyperbaric pressure [24].

The liquid ventilation technique has been employed as a controlled method to

induce hypothermia. Studies have proven this technique to offer potential as a new approach for induced hypothermia in combining the higher heat capacity of PFC liquids and the lungs as an effective heat exchanger [25,26].

Recent studies have indicated the feasibility of PFC liquids as a vehicle for pulmonary administration of drugs [27,28]. The large exchange surface of the lung and the ability of PFC liquids to distribute easily through the lung make it a promising alternative route for delivery of agents.

PFC liquids have been used clinically as radiological contrast agent for bronchography and alveolography [29]. In addition, PFC liquids are ideal substances as a contrast agent for computer tomography and magnetic resonance imaging and ultrasound technology [30].

PFC emulsions are an effective oxygen carrier to tissues and, thus, have formed a related area of interest for research as a red cell substitute [31,32]. In addition, emulsified PFC liquids have also been employed for improving tissue oxygenation in ischemic organs [33,34].

Liquid Ventilation Technique

The use of saline as a respiratory medium to investigate the pulmonary structure and function relationship, particularly the study of tissue and alveolar surface active forces [35-37], has formed the basis for the evolution of the liquid breathing concept from the saline-filled lung to ventilation with inert liquids.

As in the studies with saline-filled lung early liquid breathing studies with PFC were performed by total body immersion of spontaneously breathing animals into the

oxygenated liquid [16,38], or manually regulated gravity-assisted insufflation of animals with oxygenated PFC [39-43]. However, while adequate oxygenation could be provided, ventilation with PFC liquid did not improve CO_2 elimination and acidosis in these systems.

The inherent problem of effective CO_2 elimination related to diffusional and flow limitations associated with the high density and viscosity of the PFC liquid was avoided by employing the demand-regulated and mechanically assisted breathing systems [44-48]. Development of a modified liquid ventilator enabled liquid ventilation technique to be successfully applied in subsequent studies, even in animals with ineffective gas exchange. In this technique, extracorporeally oxygenated, warmed PFC liquid is instilled into the animal's lung at a volume equivalent to functional residual capacity volume. After degassing the lungs with postural and thoracic manipulations, the animal is connected to the liquid breathing system, and tidal liquid volumes are delivered at a frequency depending on the animal's demands while exhaled liquid is purged of CO_2 and oxygenated extracorporeally between the breaths.

Liquid Ventilation in Healthy Animals

In 1966, Clark and Gollan were the first to introduce successful PFC liquid ventilation in mice [16]. They demonstrated that, unlike animals breathing other liquids (e.g., silicone oil), animals breathing PFC liquid for several hours survived the trial and were easily reconverted to gas breathing. This work was a major advance in the field of liquid breathing offering an alternative means of respiratory medium. The longest reported liquid ventilation trial was performed in dogs and lasted only 8 hours [39]. In

various studies with liquid ventilation, pulmonary gas exchange, respiratory mechanics, cardiovascular dynamics (such as cardiac output, organ blood flow) and alveolar surface tension characteristics of healthy animal lungs were investigated. Moreover, in addition to the early changes in lung functions after liquid ventilation, the morphologic, histologic and biochemical changes have been documented in these animals in long-term follow-up studies.

PFC liquid ventilation has been demonstrated to maintain adequate oxygenation in healthy animals [40-43,49-52]. Although at satisfactory levels, PaO_2 was consistently lower in all the studies during liquid ventilation when compared to the control levels. A commonly reported issue in these experiments is the observation of increases in $PaCO_2$ and development of acidosis during liquid breathing [40-43]. The difficulty in effective elimination of CO_2 has been ascribed to the considerably high viscosity and density of the PFC liquid together with the low CO_2 diffusion coefficient [24]. Koen et al. have further explored and provided alternative ventilatory strategies to overcome this problem [47]. The changes in arterial Ph were assumed to be related to changes in $PaCO_2$ in some studies [39,41]. However, with the development of liquid breathing systems effectively eliminating CO_2, acidosis was suggested to be a distinct metabolic disturbance secondary to hyperlactatemia associated with redistribution of blood flow [50]. In a recent study, ineffective intravascular volume status was emphasized as being responsible for the metabolic acidosis [52].

Liquid ventilation usually requires high airway pressures compared to gas ventilation. Considerably high (60-100 mm Hg) peak airway pressures have been reported in isolated lung preparations during liquid ventilation [50,53]. In a study further

investigating this issue, Curtis et al. have shown that real alveolar pressures are overestimated at proximal airway pressures but mean airway pressures can be used to reflect the alveolar pressures during liquid ventilation [51].

Several researchers have reported successful reconversion to gas breathing following liquid ventilation [39-43,49,51,52]. However, a temporary impairment has been commonly observed in arterial oxygenation on returning to gas breathing [39-43,51,52] which took several days to return to preliquid breathing levels. This effect was attributed to be due to residual PFC liquid remaining in the lung that was believed to cause a diffusion defect, low ventilation/perfusion areas or decreased alveolar pO_2 [40,42,52]. Similar to changes in arterial oxygenation, reversible changes in respiratory mechanics (decrease in respiratory system compliance and increase in airway resistance) have been documented after liquid ventilation [41,42,45]. Furthermore, both increased [51] or unchanged airway pressures [39] have been reported after reconversion to gas breathing.

The effect of liquid ventilation on pulmonary surfactant and its surface tension properties was subjected to both in-vivo and in-vitro studies more than 20 years ago. One study has revealed that surfactant was not removed from the lung and the surface tension-surface area characteristics of surfactant were not altered either after liquid ventilation or lung lavage with PFC [54]. In another study, demonstrating no changes in surfactant quantity after liquid breathing, the pressure-volume relationships of isolated healthy lungs as an indirect measure of surfactant function showed a transient increase in surface active properties of surfactant [53].

Great interest centers on the effects of liquid ventilation on lung morphology and

histology. In various studies in different adult animals, hyperemia and an inflammatory reaction with macrophage infiltration were observed in the lungs only shortly after liquid ventilation [38,40], and these were suggested to correlate with the changes in blood gases [40]. However, the alveolar structures appeared to be histologically normal within few days, except that of residual vacuolated macrophages [40,49,56], and remained normal for several months [40,42,49,56] and even 3 years after liquid ventilation [21,43]. Additionally, in a morphologic study on newborn rabbits, electron microscopic data revealed that ultrastructure of the lung remained unimpaired after liquid ventilation [57].

The limited hematologic and biochemical studies suggested that no permanent deleterious effect was produced after liquid ventilation [21,56]. Despite the presence of residual PFC in all tissues, only minor changes in serum alkaline phosphatase, cholesterol, glutamic oxalacetic transaminase, glutamic pyruvate transaminase and white blood cell counts were detected, but they all returned to normal in less than 1 week following liquid ventilation.

Long-term follow-up studies have been conducted in adult animals to study the uptake, distribution and elimination of PFC liquids after various periods of liquid ventilation application. The longest reported follow-up studies, showing no adverse clinical effects, lasted approximately 3 years following 1 hr liquid ventilation [21,43]. Residual PFC was detected by gas chromatography in small amounts in all tissues, indicating systemic distribution; it was approximately 1000 times higher in the lungs and pulmonary lymph nodes than in the other tissues and, furthermore, fat contained a higher concentration of PFC than other tissues. Most importantly, residual PFC was not associated with any evidence of adverse reaction in any of the tissues. In other long-term

studies, similar results have been reported and the elimination rate of PFC from lung tissue was found to be of an exponential character and dependent on vapor pressure of PFC; the higher the vapor pressure, the faster the elimination rate [21,56,58]. Additionally, the absence of excess inorganic fluoride ions in urine supported that PFC liquids are not metabolized in biologic systems [59]. The exact mechanisms for uptake, distribution and elimination of PFC warrants further investigation while some factors such as organ blood flow, tissue lipid composition, vapor pressure of PFC, and ventilation/perfusion matching in the lung are considered to play a role.

Liquid Ventilation In Animals With Acute Respiratory Failure

To date, there are only a few reports of respiratory support with liquid ventilation in adult animals with acute respiratory failure while much of the interest has focused on preterm animals with surfactant deficiency.

The elevated surface tension at the alveolar air-liquid interface contributes to the development of pulmonary pathology (i.e., atelectasis, intrapulmonary shunting and decreased compliance) due to either surfactant deficiency as in infant RDS, or surfactant dysfunction as in adult RDS. With the hypothesis that PFC liquid would eliminate the surface forces in the surfactant deficient lung and promote alveolar stability and, thus, improve lung mechanics, Shaffer et al. employed PFC liquid ventilation in premature animals which were subject to high alveolar surface tension and structural immaturity [60]. In this and subsequent studies, Shaffer et al. showed that in comparison to gas ventilation, liquid ventilation provided better pulmonary gas exchange in preterm animals [61,62]. Moreover, these studies demonstrated that liquid-ventilated animals were

successfully reconverted to gas ventilation, and gas exchange and lung mechanics parameters were improved compared to the initial gas ventilation: PaO_2 and respiratory compliance increased, and alveolar-arterial O_2 difference and inflation pressures decreased.

Liquid ventilation was also applied to preterm animals with respiratory failure upon delivery, before initiating gas ventilation [63-65]. These studies demonstrated that liquid ventilation initiated immediately after birth provided effective pulmonary gas exchange while improving respiratory compliance and supporting cardiovascular stability.

The effect of short-term lung lavage with PFC liquid has been investigated in preterm animals and no significant changes were demonstrated in gas exchange and lung mechanics in contrast to liquid ventilation [66].

Histologic findings indicated that liquid ventilation was less harmful to the pulmonary structures when compared to gas ventilation in which hyaline membranes and epithelial necrosis were present [65,67,68]. In morphometric analysis, more homogeneously expanded and intact gas exchange units were documented in liquid-ventilated lungs in contrast to the gas-ventilated lungs [65,69].

In an adult animal model of respiratory failure, liquid ventilation with gravity-assisted technique improved pulmonary gas exchange significantly and arterial oxygenation remained higher than in the only mechanically ventilated group even after liquid ventilation was terminated [70]. In an oleic acid model of lung injury in adult animals, short periods of whole lung lavage with oxygenated PFC liquid resulted in improvements in pulmonary gas exchange and respiratory system compliance while control animals showed deterioration [71]. These beneficial effects of lung lavage with

PFC were believed to result from washing out the edema fluid and replacement of the edema fluid in the lung by the PFC with a low surface tension and high solubility for respiratory gases.

In view of the histologic and clinical data, the possible mechanisms by which liquid ventilation provided an effective therapeutic modality for treatment of respiratory failure were suggested as [65,72]: elimination of increased alveolar surface tension in liquid filled-lung, improved alveolar stability and recruitment, even distribution of pulmonary blood flow in liquid-filled lung, improved ventilation-perfusion matching resulting in decreased intrapulmonary shunting.

Clinical Investigations On Liquid Ventilation

The first human trial of PFC liquid ventilation was reported by Greenspan et al. [15]. Short periods of liquid ventilation were administered by gravity-assisted technique to three premature infants with severe respiratory failure who were considered to be clinically at the terminal stage. Although the infants eventually died of their underlying disease, marked improvements in both arterial oxygenation and respiratory compliance were observed after liquid ventilation. Since the clinical condition of the infants were not improved with other treatment modalities, these results were considered promising for future applications of this technique at an earlier stage of pulmonary dysfunction.

In parallel to animal experiments with liquid ventilation, histologic analysis of the liquid-ventilated infant lungs demonstrated uniform, well expanded lung units when compared to conventionally ventilated infants [73]. Moreover, other organs were histologically similar to those of controls.

To study the uptake, distribution, and elimination of the PFC, expired gas samples, arterial blood and various tissues were analyzed by gas chromatography in infants following liquid ventilation and were compared with controls [74]. The results indicated that PFC was rapidly eliminated by vaporization through the lungs (PFC concentration in the expired gas was within control range in 8 h), and systemic absorption and distribution of PFC to the blood and other tissues occurred while deposition was in small amounts at 2.5 months following liquid ventilation. From the limited data, it was speculated that uptake, distribution and elimination of PFC could be related to lipid composition of the tissues, perfusion of the organs, and ventilation-perfusion matching in the lung.

Potential Problems and Future Implications of Liquid Ventilation

The progress of the liquid ventilation concept from labarotory experiments for studying pulmonary physiology to human trial to restore respiratory function is overwhelming. Based on the encouraging results of animal experiments and the limited data from clinical investigational trial, liquid ventilation application has to be explored extensively in areas other than infant RDS, such as the adult RDS, aspiration syndromes, pulmonary lavage in alveolar proteinosis and cystic fibrosis as potential therapeutic considerations in clinical conditions as stated elsewhere [75]. For this purpose, a better understanding of liquid ventilation is warranted with respect to safety and efficacy in clinical practice. More importantly, before large clinical trials are undertaken, PFC liquids and liquid ventilation have to proven safe for administration for prolonged periods, and advantages over conventional therapeutic modalities have to be documented.

Although the principle of liquid ventilation is simple, the technology necessary to perform it is not encouraging. To employ liquid ventilation, there are extra technical requirements including an extracorporeal membrane oxygenator and a modified liquid ventilator. Moreover, one needs high volumes of PFC as a priming volume in liquid ventilation. At present, this complex and relatively expensive equipment has prevented liquid ventilation technique from becoming commonly and practically applied in clinical conditions. To overcome such problems, a new PFC administration technique has been explored in recent years as an alternative means of respiratory support. This new technique of partial liquid ventilation combines conventional mechanical gas ventilation and intratracheal PFC administration. In partial liquid ventilation, PFC liquid is administered into the lungs and mechanical gas ventilation is superimposed at conventional ventilator settings without necessitating an extracorporeal oxygenator and liquid ventilator.

PARTIAL LIQUID VENTILATION

The search for a simpler PFC application technique to support pulmonary gas exchange has raised the concept of partial liquid ventilation, in that conventional mechanical ventilation is combined with intratracheal PFC administration.

Partial Liquid Ventilation in Healthy Animals

Recently, Fuhrman et al. published the first report on this new technique in healthy piglets, demonstrating effective oxygenation and ventilation [17]. After filling the lungs to

the functional residual capacity volume with a PFC liquid which was fully saturated with oxygen, animals were volume-controlled ventilated for 1 h and these were compared to control animals subjected to the same ventilatory parameters with respect to gas exchange and respiratory mechanics.

In their study, arterial oxygenation was maintained about 400 mm Hg and average $PaCO_2$ was 40 mm Hg, reflecting ventilation-perfusion matching during this technique which they named as "perfluorocarbon-associated gas exchange". More importantly, the adequate gas exchange was achieved at tidal volumes and airway pressures comparable with mechanically gas ventilated animals, suggesting that the presence of PFC did not alter the mechanical function of the lung.

For the possible mechanism of action for gas exchange during partial liquid ventilation in healthy animals, the authors speculated that filling the functional residual capacity volume of the lung with PFC maintained a respiratory medium for continuous gas exchange by allowing formation of air bubbles with the superimposed tidal gas volumes. The low surface tension of PFC, as they speculated, would serve as a suitable environment for in vivo bubble oxygenation and, thus, gas diffusion from PFC to alveolar vessels would provide an effective gas exchange during partial liquid ventilation.

Partial Liquid Ventilation in Acute Respiratory Failure

Based on the observations that preterm animals could be converted to mechanical gas ventilation following liquid ventilation with favorable effects of residual PFC on gas exchange and lung mechanics, experiments were performed to investigate the effects

of intratracheal PFC administration in combination with conventional mechanical ventilation on pulmonary gas exchange in an animal model of RDS.

The first report on the effects of partial liquid ventilation in adult animals with acute respiratory failure was introduced by Tütüncü et al. [18]. After inducing RDS by repeated lung lavage with saline, PFC liquid was administered intratracheally in increasing doses (3-15 ml/kg) while animals were mechanically ventilated with volume controlled-ventilation and positive end-expiratory pressure. In contrast to isovolumic saline-treated animals, PaO_2 improved with PFC administration in a dose-dependent manner and $PaCO_2$ was maintained considerably lower. The improvement in PaO_2 with 15 ml/kg of PFC reached a level at which almost no intrapulmonary shunt occurred. The PFC doses tested were below the functional residual capacity volume (18 ml/kg) of the tested animals. Another interesting observation was that the inflation pressures were decreased to the same extent independent of the PFC dose and, thus, respiratory compliance was improved within the range of 20%-23 % at all the PFC doses. Moreover, hemodynamic stability was not impaired during this type of respiratory support.

In another study from the same group [19], intratracheal PFC administration at functional residual capacity volume was tested in animals with acute respiratory failure and the effects of 3 h partial liquid ventilation on lung functions and lung histology were compared to conventional gas ventilation and saline controls. With this technique, pulmonary gas exchange was improved and maintained stable throughout the observation period in RDS-induced adult animals (mean PaO_2 was above 400 mm Hg and mean $PaCO_2$ was below 45 mm Hg). However, the same ventilatory conditions (tidal volume and positive-end-expiratory pressure) resulted in persistent hypoxia and

hypercarbia in conventionally ventilated animals and saline-treated animals. The administration of PFC provided lung inflation at lower airway pressures (an average of 23 % less than in the untreated animals), and respiratory compliance consequently improved; these effects persisted throughout the study period. Additionally, there were marked decreases in respiratory resistance at inspiration.

Histologic data from the same study supported the clinical data and revealed that alveolar structures were well preserved with partial liquid ventilation, preventing alveolar distension and rupture. In both above-mentioned studies, metabolic acidosis was observed in PFC-treated animals despite the well preserved hemodynamic status, and was ascribed to changes in regional organ blood flow during mechanical ventilation with positive end-expiratory pressure.

These studies provided important data into the mechanisms by which PFC enhances gas exchange in the diseased lung, either during partial liquid ventilation or during gas ventilation following liquid ventilation. Moreover, the data provided physiologic implications with regard to the dissociation between the gas exchange and lung mechanics parameters during partial liquid ventilation. The dose-dependent improvement of gas exchange supported that large doses of PFC, approaching the normal functional capacity of the animal, are required to correct hypoxia as fully as possible. However, respiratory system compliance and airway pressures can be improved even with a small dose of PFC (3 ml/kg) and further doses do not make significant changes in the lung mechanics properties of lung-lavaged animals.

The responsible mechanism for the improvement in gas exchange during partial liquid ventilation was suggested to be mainly due to the filling of the alveoli with PFC

liquid so that the more PFC applied the more collapsed alveoli could be opened up and prevented from end-expiratory collapse. Thus, the lung volume participating in effective gas exchange would increase in parallel with the increasing PFC doses, thereby eliminating intrapulmonary shunt. The high solubility of O_2 and CO_2 in PFC would also contribute to the continuous pulmonary gas exchange in this system. This mechanism of action for PFC was confirmed by the well maintained PaO_2 for several hours, as well as by the histologic findings.

By contrast, the remarkably low surface tension of PFC liquid accounted for the changes in the mechanical behaviors of the respiratory system independent of the PFC dose during partial liquid ventilation. The changes in respiratory mechanics suggested that, following administration of even a low dose of PFC (3 ml/kg), a thin film of PFC with a low surface tension is formed due to the evaporation and covers the lung units in the whole lung. This hypothetical film of PFC reduces the elevated surface tension at the alveolar air-liquid interface in the diseased lung. Therefore, the reduction of surface tension by PFC facilitates easy lung expansion (improves respiratory compliance) during partial liquid ventilation. It follows that once the surface tension is reduced to the level of acting PFC, additional PFC doses would not cause further changes in the mechanical behavior of the lung.

These studies demonstrated the feasibility of partial liquid ventilation in improving pulmonary gas exchange and respiratory mechanics in animals with RDS and, in this regard, this new type of ventilatory support proved to offer advantages over conventional mechanical ventilation in the short term. Considering the remarkable reductions in airway pressures during partial liquid ventilation while identical ventilation parameters induced

barotrauma during conventional ventilation, this new technique appears to be an alternative modality to minimize or prevent the progress of lung injury. Moreover, the data support the importance of a low surface tension at the air-liquid interface in the lung. Furthermore, as stated elsewhere [76], this technique holds major advantages compared with total liquid ventilation such as requirement of less PFC and ease of application without necessitating extra technology (extracorporeal oxygenator, modified liquid ventilator).

Future Investigations and Implications

Consideration of another ventilatory support modality for clinical use in critical care requires documentation of an advantage compared with available techniques. The advantage should be evident in one or more of the following areas: safety, expense, ease of operation, or therapeutic outcome.

At present, partial liquid ventilation is confined to only a few animal experiments and the available data are limited to short-term (3 h) effects on pulmonary gas exchange, respiratory mechanics and hemodynamic variables. In addition to long-term administration, efficacy in other models of acute respiratory failure has to be investigated as an alternative treatment modality. With respect to its safety and tissue toxicity, further studies are required, although the limited data on tissue toxicity have proven PFC to be safe both in animal studies with liquid ventilation [21] and in clinical trials as a contrast agent [77].

Another point for further investigation is the development of metabolic acidosis and hemodynamic compromise. Since PFC liquids are dense liquids, the effects of

intratracheal PFC administration on cardiopulmonary interaction needs to be investigated as a contributory factor for altered organ blood flow and occurrence of lactic acidosis, although intravascular fluid volume plays an important role in maintaining adequate oxygen supply during liquid ventilation [52].

All types of mechanical ventilation modalities are means of supportive treatment until the primary disorder is cured. When the long-term application of partial liquid ventilation is considered, repetitive doses of PFC will be inevitably required due to the evaporation. In this respect, cost effectivity of this type of ventilatory support needs to be further investigated and compared with other treatment modalities, although this point makes partial liquid ventilation advantageous over liquid ventilation.

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CHAPTER 2

INTRATRACHEAL PERFLUOROCARBON ADMINISTRATION COMBINED WITH MECHANICAL VENTILATION IN EXPERIMENTAL RESPIRATORY DISTRESS SYNDROME: DOSE-DEPENDENT IMPROVEMENT OF GAS EXCHANGE

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INTRATRACHEAL PERFLUOROCARBON ADMINISTRATION COMBINED WITH MECHANICAL VENTILATION IN EXPERIMENTAL RESPIRATORY DISTRESS SYNDROME: DOSE-DEPENDENT IMPROVEMENT OF GAS EXCHANGE

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ABSTRACT

Objectives: To test the efficacy of intratracheal instillation of a perfluorocarbon, combined with conventional mechanical ventilation, as well as to establish the dose response of this application on pulmonary parameters in adult animals with acute respiratory failure.

Design: Prospective, randomized, placebo-controlled study.

Setting: Anesthesiology laboratory of a university.

Subjects: Twelve, adult male New Zealand rabbits.

Interventions: After inducing respiratory failure by repeated lung lavage with saline, one group of animals was treated with perfluorocarbon, while another group was treated with saline to serve as controls (n = 6 per group). Treatment consisted of intratracheal instillation of incremental doses of 3 mL/kg of each liquid up to a total volume of 15

mL/kg. Animals were mechanically ventilated for 15 minutes after each treatment dose with volume-controlled ventilation, a tidal volume of 12 mL/kg, frequency of 30 breaths/min, FiO_2=1.0, and positive end-expiratory pressure of 6 cm H_2O.

Measurements and Main Results: Arterial blood gases and lung mechanics were determined. In the perfluorocarbon group, PaO_2 increased with increases in dosage from 75 ± 15 to 420 ± 27 torr (10.0 ± 2.0 to 55.9 ± 3.6 kPa); $PaCO_2$ decreased from 49 ± 6 to 43 ± 5 torr (6.5 ± 0.8 to 5.7 ± 0.6 kPa) after first dose, and remained stable thereafter. Airway pressures were significantly lower after treatment compared to pretreatment values.

Conclusion: The remarkable improvements in pulmonary parameters suggest that this type of ventilatory support offers an effective and simple method of perfluorocarbon application in acute respiratory failure.

INTRODUCTION

Although recent advances in conventional ventilation have improved the survival rates for patients in respiratory distress syndrome (1,2), this syndrome is one of the major problems in intensive care units because of its complex pathogenesis. The predominant characteristics of respiratory distress syndrome include hypoxemia due to intrapulmonary shunting of blood, and decreased lung compliance due to impaired or deficient surfactant function (3-5).

Oxygenated perfluorocarbon liquids have been employed as an alternative respiratory medium in surfactant-deficient lungs due to their low surface tension property

and their ability to dissolve large volumes of respiratory gases. Perfluorocarbon liquid ventilation improved lung mechanics and distribution of pulmonary gas exchange in animal studies (6-11), particularly in surfactant-deficient preterm animals.

The administration of high volumes of perfluorocarbon liquids and the necessity of a modified liquid-breathing system have raised the issue of practicality of liquid ventilation. We hypothesized that intratracheal instillation of perfluorocarbon liquids also could be a therapeutic measure in combination with conventional mechanical ventilation to improve pulmonary gas exchange in acute respiratory failure. To test this hypothesis, we performed a study investigating the effects of intratracheal instillation of small amounts of Perflubron (Alliance Pharmaceutical, San Diego, CA) (perfluorooctylbromide), a perfluorocarbon with a low surface tension (18.1 dyne/cm), in combination with conventional mechanical ventilation on respiratory parameters in an adult animal model of acute respiratory failure.

MATERIALS AND METHODS

Animal Preparation: This study was approved by the Animal Committee of the Erasmus University of Rotterdam.

Twelve adult New Zealand rabbits(2.8 ± 0.2 kg) were anesthetized with an intravenous injection of pentobarbital sodium (50 mg/kg) via an auricular vein. The animals were positioned supine, tracheotomized and an endotracheal tube was inserted midway along the trachea, with its tip proximal to the carina, after which, mechanical ventilation with a Servo Ventilator (900C, Siemens-Elema, Solna, Sweden) was initiated

using an FiO_2 of 1.0 and zero end-expiratory pressure with a constant tidal volume of 12 mL/kg, a frequency of 30 cycles/min, and inspiratory to expiratory ratio of 1:2. Anesthesia was maintained with additional doses of pentobarbital sodium, as required; pancuronium bromide was administered as a bolus (0.1 mg/kg iv), followed by a continuous infusion (0.1 mg/hr/kg) for muscle paralysis. Via the auricular vein, 5% dextrose/0.45% sodium chloride solution was continuously administered at a rate of 15 mL/h as a maintenance fluid. No additional drug treatment (including bicarbonate) was given. A heating blanket maintained core temperature at 37 ± 1°C, monitored by an esophageal thermistor (Elektrolaboratoriet, Copenhagen, Denmark).

A femoral artery and femoral vein were cannulated with polyethylene catheters for arterial and central venous pressure recording and blood sampling. A special indwelling catheter (Mikro-pO2-Messkatheter, Licox, Keil-Mielkendorf, FRG) was inserted into the other femoral artery for on-line oxygen pressure monitoring (Licox). Arterial blood gases, pH, and hemoglobin concentrations were determined by conventional methods (ABL-330 and Osm-2 Hemoximeter, Radiometer, Copenhagen, Denmark). Lung mechanics (airway pressures, compliance, and airway resistance) were measured on-line by means of a calculator (Lung Mechanics Calculator 940, Siemens-Elema), which has been shown to be a reliable and accurate calculator both for infants and adults (12). End-tidal CO_2, deadspace ventilation, alveolar ventilation, and CO_2 production rates were measured on-line by means of a CO_2 analyzer (930, Siemens). Intravascular pressure monitoring was made using transducers (P23XL, Spectramed, Oxnard, CA) that were zero-referenced to midchest level, and all tracing, including electrocardiogram, were traced by a monitor (Sirecust 1280, Siemens), and recorded by a recorder (Siredoc 220, Siemens).

Model of Respiratory Insufficiency: After baseline observations, lung lavage with 30 mL/kg warm saline (37°C) was performed in all animals (13), after which, positive end-expiratory pressure was increased to 6 cm H_2O. Lung lavage was repeated four to six times to achieve a PaO_2 of <100 torr (<13.3 kPa) at the following ventilator settings: volume-controlled ventilation; FiO_2=1.0; tidal volume = 12 mL/kg; frequency = 30 cycles/min; inspiratory/expiratory ratio = 1:2; and a positive end-expiratory pressure of 6 cm H_2O.

Treatment: Perflubron is a perfluorocarbon that is insoluble in water and that is very stable, without any reaction with air or water at normal temperatures and pressures. Perflubron has a specific gravity of 1.918 g/cm^3 at 25°C, a surface tension of 18.1 dynes/cm, vapor pressure of 3.6 torr (0.5 kPa) at 20°C and 10.5 torr (1.4 kPa) at 37°C, an oxygen solubility of 53 mL/100 mL and CO_2 solubility of 210 mL/100 mL at 37°C and 1 atmosphere pressure.

After induction of respiratory insufficiency, animals were divided randomly into two groups. Group 1 (n = 6) was treated with perfluorocarbon. Group 2 (n = 6) received saline and served as controls. Treatment consisted of intratracheal instillation of incremental doses of 3 mL/kg of each liquid up to a total volume of 15 mL/kg. At instillation, animals were disconnected from the ventilator and treatment liquid (warmed to room temperature) was administered directly into the endotracheal tube over 3 to 5 secs; the ventilator was then reconnected immediately. After each treatment dose, animals were mechanically ventilated for 15 mins to achieve a steady state with the above-mentioned settings. Cardiocirculatory parameters, lung mechanics, CO_2 gas exchange, arterial blood gases, and hemoglobin were determined at 15-min intervals

after each treatment. Animals were killed by administration of a high dose of pentobarbital after the last measurement.

Statistical analysis: All data are presented as mean ± SD. The data were assessed by the Mann-Whitney U test for intergroup comparison and by the Wilcoxon signed-rank test for intragroup comparisons. A $p < .05$ was considered statistically significant.

RESULTS

Lung lavage induced an acute respiratory failure in all animals, as demonstrated by a decrease in PaO_2 and an increase in airway pressures. All animals survived the experiment, over a period of 80 mins. The initial arterial blood gas values (pre and postlavage) were comparable in both groups (Figure 1, top left). PaO_2 increased significantly ($p < .05$) with each subsequent dose of perfluorocarbon, from a pretreatment value of 75 ± 15 to 420 ± 27 torr (10.0 ± 2.0 to 55.9 ± 3.6 kPa) at the end; Figure 1 (top left) represents the dose-response trend in PaO_2. PaO_2 levels after the doses of 12 and 15 mL/kg were not statistically significant when compared with the prelavage value. $PaCO_2$ decreased significantly ($p < .05$) from 49 ± 6 to 43 ± 5 torr (6.5 ± 0.8 to 5.7 ± 0.6 kPa) after instillation of 3 mL/kg perfluorocarbon and remained stable throughout the experiment (Figure 1, top right). After the first dose, arterial pH improved in parallel with a decrease in $PaCO_2$ in the perfluorocarbon group and remained within the range of 7.34 to 7.31 thereafter (Figure 1, bottom right). In contrast to perfluorocarbon group, the data for PaO_2, $PaCO_2$, and pH showed gradual deterioration

in the saline group.

The peak airway pressure required to mechanically ventilate lungs with constant tidal volumes decreased significantly from 25.5 ± 1.0 cm H_2O to 21.3 ± 1.4 cm H_2O after administration of 3 mL/kg of perfluorocarbon and remained stable with the consequent treatment doses (Figure 1, bottom left). There was also a decrease in mean airway pressure in the perfluorocarbon group (Table 1). Inspiratory airway resistance was observed to decrease significantly in the perfluorocarbon group compared with pretreatment values. Expiratory airway resistance also decreased after treatment with perfluorocarbon (from 117 ± 0.7 to 107 ± 10 cm H_2O/L/sec), although this decrease did not reach statistical significance. Neither airway pressures nor airway resistances changed in the saline group. After treatment with perfluorocarbon, lung compliance improved significantly; there were no more statistically significant changes after 9 and 12 mL/kg treatment doses compared with the prelavage value (Table 1).

After 3 mL/kg of perfluorocarbon administration, alveolar ventilation was well preserved; alveolar dead space was lowered and remained stable with subsequent doses (Table 2). CO_2 gas exchange, which is determined by the metabolic rate and reflected by CO_2 minute production, was stable during treatment with perfluorocarbon, indicating a well-preserved metabolic activity. CO_2 minute production decreased significantly in the saline group, which is likely to be due to impaired aerobic metabolism resulting from severe hypoxia (Table 2).

The cardiocirculatory status of the treatment groups is shown in Table 3. The hemodynamic data were comparable before and after inducing respiratory failure in both groups. All animals in the perfluorocarbon group tolerated the treatment well without

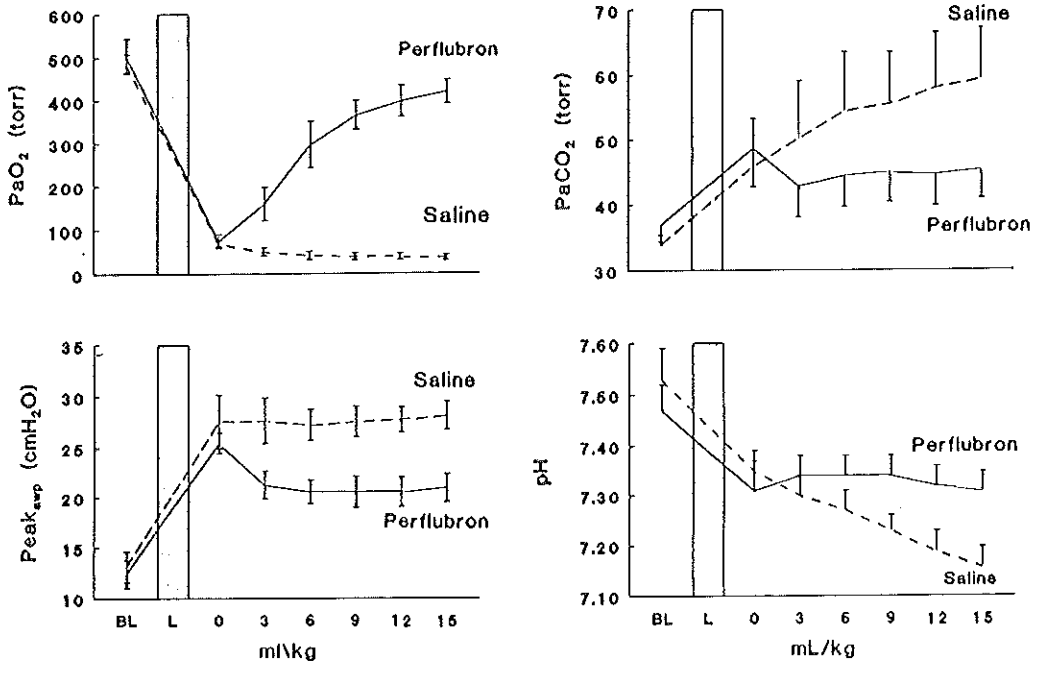

Figure 1. *Top left:* PaO_2 (mean ± SD, 8 torr = 1 kPa) before lavage (BL), after lavage (0), and after treatment with perfluorocarbon or saline. The bar represents the lavage procedure (L). After the lung lavage, PaO_2 shows significant decrease in both groups. Mean PaO_2 increases significantly ($p < .05$, Wilcoxon signed-rank test) with each increasing dose of perfluorocarbon, showing no statistical significance after 12 mL/kg, while, in the saline group, oxygenation is severely impaired at the same ventilator settings. *Top right:* Changes in $PaCO_2$ (mean ± SD, 8 torr = 1 kPa) after intratracheal instillation of perfluorocarbon or saline in combination with mechanical ventilation for 15 min. When compared with pretreatment value (0 point), $PaCO_2$ is lower in the perfluorocarbon group at all the treatment doses and significantly differs from the saline group after 6 mL/kg dose onward. *Bottom right:* Arterial pH is seen to decrease in both groups subsequent to lung lavage (L). This decrease is followed by further significant decreases in the saline group, but arterial pH remains stable in the perfluorocarbon group. *Bottom left:* Peak airway pressures ($Peak_{awp}$) before lavage, after lavage (0), and in response to perfluorocarbon or saline administration (mean ± SD). Positive end-expiratory pressure of 6 cm H_2O was applied during lavage procedure according to the experimental protocol. Individual animal data in the perfluorocarbon group showed significant ($p < .05$) decreases in peak airway pressures after 3 mL/kg- between 18% and 23% (average 20.3 ± 2.5%)- when compared with pretreatment values. Mean data are significantly ($p < .005$, Mann-Whitney U test) different from the saline group at all treatment doses.

Table 1: Lung mechanics parameters of perfluorocarbon-treated and saline-treated animals (mean ± SD)

	$Mean_{awp}$ (cm H_2O)		R_i (cm H_2O/L/sec)		C_{rs} (mL/cm H_2O)	
	Perfluorocarbon	Saline	Perfluorocarbon	Saline	Perfluorocarbon	Saline
Prelavage	3.3 ± 0.3	3.5 ± 0.4	23.7 ± 3.1	19.0 ± 3.1	3.39 ± 0.78	2.87± 0.25
Postlavage	11.3 ± 0.4	11.7 ± 0.8	41.5 ± 3.3	35.5 ± 3.3	1.90 ± 0.09	1.71 ± 0.14
3 ml/kg	10.1 ± 0.2[a,b]	11.9 ± 0.5	37.7 ± 3.2	33.7 ± 3.5	2.50 ± 0.14[a,b]	1.67 ± 0.14
6 ml/kg	10.0 ± 0.2[a,b]	12.0 ± 0.6	33.5 ± 3.8[b]	34.0 ± 3.3	2.56 ± 0.08[a,b]	1.66 ± 0.19
9 ml/kg	9.9 ± 0.1[a,b]	12.1 ± 0.4	32.8 ± 3.5[b]	32.8 ± 3.1	2.58 ± 0.14[a,b]	1.62 ± 0.13
12 ml/kg	10.0 ± 0.2[a,b]	12.2 ± 0.5	32.7 ± 3.8[b]	33.3 ± 4.3	2.53 ± 0.07[a,b]	1.59 ± 0.13
15 ml/kg	10.2 ± 0.2[a,b]	12.4 ± 0.5	31.8 ± 3.8[b]	33.5 ± 4.8	2.36 ± 0.09[a,b]	1.59 ± 0.14

$Mean_{awp}$, mean airway pressure; R_i, inspiratory airway resistance; C_{rs}, respiratory system compliance;
a = $p < .01$ compared with the saline-treated group by Mann-Whitney U test.
b = $p < .05$ compared with postlavage value by Wilcoxon signed-rank test.

Table 2: Gas exchange and ventilatory parameters of the treatment groups (mean ± SD)

	$ETCO_2$ (%)		Vco_2 (mL/min)		V_A (L/min)		V_D (mL)	
	Perfluorocarbon	Saline	Perfluorocarbon	Saline	Perfluorocarbon	Saline	Perfluorocarbon	Saline
Prelavage	3.26 ± 0.3	3.01 ± 0.2	19.5 ± 3.2	21.5 ± 3.6	0.72 ± .04	0.75 ± .16	6.7 ± 1.5	6.5 ± 0.5
Postlavage	3.46 ± 0.2	3.31 ± 0.4	17.3 ± 2.3	16.3 ± 2.7	0.58 ± .10	0.67 ± .13	10.2 ± 1.0	11.0 ± 0.9
3 ml/kg	3.39 ± 0.2	3.32 ± 0.4	18.0 ± 1.8	15.7 ± 2.3	0.58 ± .08	0.63 ± .15	9.3 ± 0.5[a]	11.2 ± 1.2
6 ml/kg	3.41 ± 0.3	3.34 ± 0.4	17.8 ± 2.1	15.0 ± 2.2	0.60 ± .09	0.62 ± .17	9.0 ± 0.9[a]	11.2 ± 1.0
9 ml/kg	3.44 ± 0.3	3.19 ± 0.3	18.0 ± 2.1[a]	14.3 ± 2.0[b]	0.62 ± .08	0.60 ± .11	9.3 ± 0.8[a]	11.5 ± 1.0
12 ml/kg	3.44 ± 0.3	3.15 ± 0.3	18.0 ± 1.8[a]	13.8 ± 2.0[b]	0.60 ± .09	0.55 ± .15[b]	9.2 ± 0.8[a]	11.7 ± 1.0[b]
15 ml/kg	3.51 ± 0.4[a]	3.04 ± 0.2	17.5 ± 2.1[a]	13.8 ± 1.7[b]	0.55 ± .05	0.53 ± .10[b]	10.2 ± 1.2[a]	12.0 ± 1.1[b]

$ETCO_2$, end-tidal CO_2 concentration; Vco_2, CO_2 production; V_A, alveolar ventilation; V_D, physiologic deadspace.
a = $p < .05$ compared with the saline-treated group by Mann-Whitney U test.
b = $p < .05$ compared with the postlavage value by Wilcoxon signed-rank test.

Table 3: Hemodynamic variables of perfluorocarbon-treated and saline-treated animals (mean ± SD)

	MAP (mm Hg)		HR (beats/min)		CVP (mm Hg)	
	Perfluorocarbon	Saline	Perfluorocarbon	Saline	Perfluorocarbon	Saline
Prelavage	92 ± 14	96 ± 9	338 ± 33	345 ± 19	5 ± 0.5	4 ± 0.8
Postlavage	83 ± 17	90 ± 13	335 ± 35	330 ± 21	5 ± 1.0	4 ± 0.9
3 ml/kg	87 ± 9	86 ± 8	333 ± 40	330 ± 21	4 ± 1.2	4 ± 0.7
6 ml/kg	87 ± 4[a]	76 ± 5	330 ± 27[a]	310 ± 18	4 ± 0.9	4 ± 0.6
9 ml/kg	85 ± 5[a]	77 ± 4	325 ± 30[a]	295 ± 8[b]	4 ± 1.1	4 ± 0.7
12 ml/kg	86 ± 7[a]	77 ± 4[b]	320 ± 28[a]	288 ± 15[b]	4 ± 0.9	4 ± 0.7
15 ml/kg	85 ± 9[a]	73 ± 7[b]	308 ± 30[a]	268 ± 11[b]	5 ± 1.0	4 ± 0.7

MAP, mean arterial pressure; HR, heart rate; CVP, central venous pressure.
a = $p < .05$ compared with the saline-treated group by Mann-Whitney U test.
b = $p < .05$ compared with postlavage value by Wilcoxon signed-rank test.

significant changes in mean arterial pressure (MAP), heart rate (HR), and central venous pressure (CVP). Meanwhile, MAP showed significant decreases in the saline group with accompanying significant decreases in HR during the course of the experiment.

DISCUSSION

This article reports the first experimental data related to intratracheal perfluorocarbon administration in combination with conventional mechanical ventilation (partial liquid ventilation) to improve pulmonary gas exchange in a dose-dependent manner in an animal model of adult respiratory failure. With respect to stability, easy control of the severity, and reproducibility of the damage, this model of lung injury is favorable in comparison to other models of adult respiratory distress syndrome (ARDS),

such as infusion of endotoxin or bacteria, infusion of oleic acid, and hydrochloride instillation (14). Although the lung injury generated in this model in the early stage is more uniform and resembles neonatal respiratory distress syndrome rather than ARDS, in late stages, it represents the pathophysiologic and morphologic features of severe ARDS (increased alveolar permeability, atelectasis, decreased compliance, protein-rich pulmonary edema) and has been extensively used in experimental studies (13-18).

Perfluorocarbon liquid ventilation has been investigated extensively in animals. It has been demonstrated to improve pulmonary gas exchange in preterm animals with respiratory failure and to maintain adequate oxygenation in healthy adult animals (6-11, 19-22). The first clinical application of this technique has been attempted in prematurely born neonates with some promising results by Greenspan et al (23).

Up to now, liquid ventilation for treatment of respiratory failure has been performed by extracorporeally oxygenated perfluorocarbon liquid, after instillation of perfluorocarbon in a quantity to replace the gas volume of functional residual capacity. To achieve this approach, one needs a modified ventilator in combination with an extracorporeal oxygenator (24). Our approach in this experiment differed in two ways from that approach in liquid ventilation. First, animals were subjected to a conventional mechanical ventilator with volume-controlled ventilation throughout the experiment without necessitating extra technical equipment or instrumentation. Second, we used liquid volumes starting from amounts below the volume of functional residual capacity and increased the amount to investigate whether there is a dose-response effect between the applied perfluorocarbon volume and respiratory parameters. Using a conventional ventilator and replacing the entire functional residual capacity volume with

perfluorocarbon, Fuhrman et al. (25) recently reported the first data with this new technique, showing adequate ventilatory support in healthy animals.

We observed a dose-dependency for PaO_2 but not for the mechanical behaviors of the lung (e.g., lung compliance). In other words, PaO_2 was the only measured parameter that continued to change after the administration of 3 mL/kg of perfluorocarbon. This treatment dose is far below the functional residual capacity volume, which has been measured to be 8.6 ± 1.0 mL/kg in lung-lavaged rabbits and 18.4 ± 2.7 mL/kg in healthy rabbits at identical conditions (an average body weight of 2.7 kg and positive end-expiratory pressure of 6 cm H_2O) (26). The action of perfluorocarbon liquid as observed in the present study suggests that when perfluorocarbon liquid is administered into diseased lungs, it acts by different mechanisms for lung mechanics and oxygenation.

We speculate that due to evaporation of perfluorocarbon, the whole lung is lined with perfluorocarbon at the alveolar air-liquid interface after administration of the initial perfluorocarbon dose. The low surface tension of perfluorocarbon eliminates the high surface tension at the air-liquid interface in the surfactant deficient lung, and alveolar expansion is thus facilitated at much lower airway pressures, and the lung behaves almost as a fluid-filled lung (27). Our experimental results support the assumption that once the surface tension is reduced to the level associated with the administered perfluorocarbon liquid, additional doses would not provide further reductions in airway pressures but should also avoid increases in airway pressures as long as the lungs are ventilated in a region (lung volume) where tissue elasticity does not play a major role (27).

The dose-dependent improvement in oxygenation requires another approach to define the possible mechanism of perfluorocarbon, with particular focus on physiologic functions of lung surfactant.

One of the major properties of lung surfactant is the ability to change surface tension in relation to the alveolar radius (e.g., to reduce surface tension to nearly zero at end-expiration). This main characteristic of lung surfactant ensures that, at very low lung volumes, alveoli are kept open on one side, and on the other side, alveolar stability is maintained independent of radius. In contrast to lung surfactant, perfluorocarbons have constant, relatively low surface tensions (for Perflubron, it is 18.1 dyne/cm) that are independent of the surface area (respectively volume). Therefore, treatment of a lung with perfluorocarbon enables an easy inspiration, but can not prevent alveoli from end-expiratory collapse in a surfactant-deficient lung. This situation means that perfluorocarbon can replace normal surfactant only in one function, but it can not neutralize the action of the law of Laplace in surfactant-deficient alveoli, so that the pressure in an alveolus will depend only on the radius because the surface tension of perfluorocarbon is constant: pressure = 2 x surface tension/radius. In other words, to keep a small alveolus open at end-expiration, one needs much higher pressures. Thus, from a physical point of view, perfluorocarbon cannot replace natural surfactant.

Due to the fact that the level of PaO_2 depends on the volume of perfluorocarbon administered into the lung, we speculate that the dose dependency of PaO_2 is related to the number of perfluorocarbon-filled alveoli in which gas exchange can also take place during expiration. The nonfilled alveoli, presumably in the non-dependent (upper) parts of the lung, would collapse during end-expiration and, thus, increase intrapulmonary

shunt as demonstrated by the low PaO_2 levels at small doses of perfluorocarbon. By replacing the volume of functional residual capacity with perfluorocarbon, the affected alveoli can no longer collapse. The more liquid that is administered, the more alveoli will be prevented from end-expiratory collapse; additionally, the large capacity for dissolved oxygen and CO_2 of perfluorocarbon helps maintain a respiratory medium for continuous alveolar gas exchange (e. g. almost no shunt occurs). This finding was confirmed by our experimental results with a PaO_2 of 420 ± 27 torr (55.9 ± 3.6 kPa).

This hypothesis of action of perfluorocarbon in surfactant-depleted lungs is indirectly supported by a surfactant replacement study (26) in lung-lavaged rabbits, which demonstrated that administration of a surfactant suspension volume of 3 mL/kg (40 mg phospholipids/mL) on one side increased PaO_2 to >500 torr (>66.5 kPa). However, on the other side, peak airway pressure was higher in the surfactant-treated group in comparison with the perfluorocarbon-treated group of the present study, which can be explained by the higher static surface tension of the surfactant preparation tested (about 23 dynes/cm).

In findings that are similar to the present experimental data, improvements in lung mechanics, such as decreases in peak airway pressures and increases in lung compliance, have been documented after reconversion to gas breathing in liquid ventilation studies (6-8, 10) in animals with respiratory distress syndrome. These improvements have been ascribed to the remaining perfluorocarbon liquid in the lungs.

In contrast to the difficulties in effective elimination of CO_2 reported during liquid ventilation studies (28,29), our results suggest that perfluorocarbon liquids do not impose limitations for effective CO_2 elimination with this new type of ventilatory support in acute

respiratory distress syndrome in animals. Although better ventilation/perfusion matching has also been reported during liquid ventilation in comparison to gas ventilation in animals with respiratory distress syndrome, our approach has accomplished this goal with less amount of perfluorocarbon. In liquid ventilation, elimination of CO_2 is determined by several factors, including diffusion and flow limitations in perfluorocarbon liquids, and studies (29,30) have investigated the means of overcoming this inherent problem, suggesting readjustments in the ventilatory pattern during liquid ventilation. In contrast to liquid ventilation, these limiting factors do not play a role in this form of ventilatory support. Therefore, this technique of partial liquid ventilation offered effective CO_2 elimination and, from this point of view, also may have some advantage over total liquid ventilation.

Intratracheal perfluorocarbon instillation did not cause significant changes in the hemodynamic status. There were slight decreases in MAP in both groups after lung lavage that could be attributed to decreased venous blood return due to relatively high inflation pressures and the applied positive end-expiratory pressure. After treatment with perfluorocarbon, MAP reached baseline values and remained stable, while, in the saline-treated group, MAP decreased further during the course of the treatment, presumably due to the accompanying hypoxia, which also was demonstrated by the HR changes.

During application of this supportive treatment, one has to consider preventing the movement of perfluorocarbon liquid along the airways. From this point of view, the level of positive end-expiratory is important to prevent the bulk movement of liquid, especially when high volumes are administered. The observation that supports this idea is that there were airway pressure changes, traced from the pressure recordings, immediately

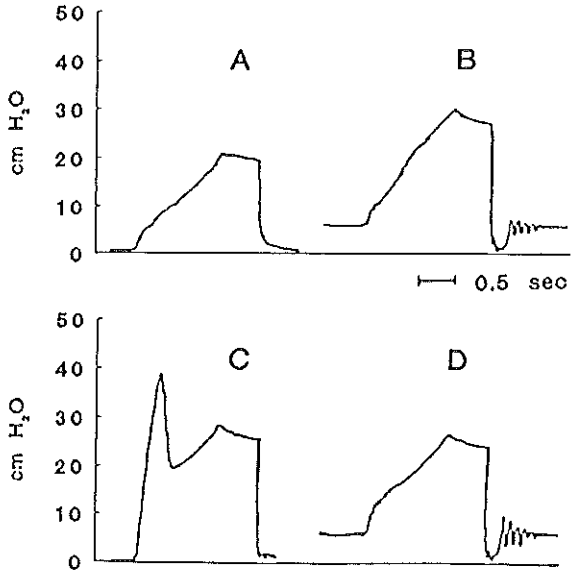

Figure 2. Inspiratory pressure tracings recorded from an animal in the perfluorocarbon group at different phases of the experiment. A) Normal pattern before lung lavage (0 positive end-expiratory pressure); B) After inducing respiratory failure, before treatment (positive end-expiratory pressure = 6 cm H_2O); C= Just after intratracheal administration of 15 mL/kg perfluorocarbon (0 positive end-expiratory pressure); D) A few breaths after administration of 15 mL/kg perfluorocarbon (positive end-expiratory pressure = 6 cm H_2O). As can be seen from the inspiratory pressure tracing in C, there was instantaneous increase in airway pressure (traced as a spike), which resolved within a few cycles of breath until the applied positive end-expiratory pressure was set by the ventilator.

after reconnection to the ventilator after the instillation of high doses of perfluorocarbon liquid into the trachea. This transient phenomenon is attributed to the backward and forward movement of the previously instilled liquid due to the discontinuation of positive end-expiratory pressure and/or to the bulk movement of the administered liquid until the positive end-expiratory pressure, which keeps the perfluorocarbon liquid in the peripheral airways, is set by the ventilator (Figure 2).

Our finding that this type ventilatory support does no harm to the lungs is supported by the study of Fuhrman et al (25). They demonstrated that this type of ventilatory support could provide adequate gas exchange in healthy animals at airway pressures comparable with continuous positive-pressure ventilation, and, furthermore, there were no deleterious effects of filling healthy lungs with perfluorocarbon liquid using this new technique.

This present study demonstrated that pulmonary gas exchange can, in the short-term, be improved in a dose-dependent manner with partial liquid ventilation in adult animals with acute respiratory failure. Moreover, significant reductions can be achieved in the airway pressures needed to ventilate the lungs with the same volumes of gas. The simplicity of this application, combined with the reduced amounts of perfluorocarbon liquid needed compared with liquid ventilation, could be of importance for clinical application of this new supportive method, both from a cost perspective and from the viewpoint of the ease of application in intensive care units. In addition, its low vapor pressure makes Perflubron the choice of perfluorocarbon for this type of ventilatory support when the evaporative losses have to be taken into consideration.

Finally, it is important to emphasize that further studies are necessary to establish the long-term efficacy, hemodynamic stability, and nontoxicity of this new treatment modality before suggesting clinical use. Meanwhile, no evidence of tissue damage was revealed by either the toxicology studies on Perflubron used in clinical trials as a contrast agent for computed tomography and magnetic resonance imaging (31), or by the limited data on long-term toxicity of perfluorocarbon in liquid-breathing studies (32) in animals.

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CHAPTER 3

COMPARISON OF VENTILATORY SUPPORT WITH INTRATRACHEAL PERFLUOROCARBON ADMINISTRATION AND CONVENTIONAL MECHANICAL VENTILATION IN ANIMALS WITH ACUTE RESPIRATORY FAILURE

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COMPARISON OF VENTILATORY SUPPORT WITH INTRATRACHEAL PERFLUOROCARBON ADMINISTRATION AND CONVENTIONAL MECHANICAL VENTILATION IN ANIMALS WITH ACUTE RESPIRATORY FAILURE

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ABSTRACT

We investigated the efficacy of intratracheal perfluorocarbon (PFC) administration combined with mechanical ventilation to support gas exchange in adult animals with acute respiratory failure. These were compared with a similar group of animals treated with continuous positive pressure ventilation (CPPV) with respect to respiratory parameters and postmortem lung histology. After lung lavage with saline, 18 adult rabbits were divided into three groups (n = 6 per group). All groups received conventional CPPV. Additionally, one group received intratracheal PFC administration at a volume of 18 ml/kg (corresponding to functional residual capacity) (PFC group); another received 18 ml/kg of saline administration (saline group), and the last had no further treatment (CPPV group). All groups were ventilated for 3 h using 100% oxygen, with the same ventilator settings of tidal volume of 12 ml/kg, respiratory frequency of 30/min, and

positive end-expiratory pressure of 6 cm H_2O. In the PFC group, PaO_2 increased from 67.2 ± 11.4 to 424.2 ± 14 mm Hg and remained stable for 3 h with well-preserved $PaCO_2$ values. Only in the PFC group were significant decreases in airway pressures and increase in respiratory system compliance seen. In the CPPV group, PaO_2 stayed around 60 mm Hg and $PaCO_2$ gradually increased. PFC treatment with conventional mechanical ventilation in acute respiratory failure proved to be a successful supportive technique to improve gas exchange at low inflation pressures.

INTRODUCTION

The main objectives of clinical management of respiratory distress syndrome (RDS) are supply of adequate arterial and tissue oxygenation and treatment of the primary disorder. To achieve the first goal, different mechanical ventilation modalities with supplemental oxygen have been introduced with particular emphasis on avoiding further lung injury as a result of mechanical ventilation (1-6).

In recent years, perfluorocarbon (PFC) liquid ventilation has been demonstrated to be effective in improving pulmonary gas exchange and thus management of RDS in animals, even when conventional ventilation was insufficient to support life (7-10). These experiments led to the first clinical application of liquid ventilation in preterm neonates by Greenspan and colleagues (11). Measures to increase arterial oxygenation have been technically successful in liquid ventilation, and this has led to investigations designed to simplify methods of PFC application, combining conventional mechanical ventilation and intratracheal PFC administration. Fuhrman and colleagues recently demonstrated that

adequate pulmonary gas exchange can be provided in healthy animals with this new technique, after filling the lungs to functional residual capacity (FRC) volume with PFC (12). Using this new type of respiratory support, we observed PFC dose-dependent (from 3 ml/kg to 15 ml/kg) improvements in pulmonary gas exchange and improvements in lung functions in animals with acute respiratory failure (unpublished observations). The present study was designed to investigate the effects on lung functions and lung histology of this neMwtreatment modality using large PFC doses. Comparisons with conventional gas ventilation and a liquid (saline) control were made during a 3-h observation period in an acute respiratory failure model.

METHOD

This study was approved by the Animal Care Committee of Erasmus University Rotterdam and was designed to be a prospective randomized trial in 18 animals following induction of respiratory failure.

Animal Preparation

A total of 18 adult New Zealand rabbits with a mean body weight of 2.8 ± 0.2 kg (range 2.4 to 3.2 kg) were anesthetized with intravenous pentobarbital sodium (50 mg/kg) via an auricular vein. Tracheostomy was performed, and a 3.0 to 3.5 mm inner diameter endotracheal tube was introduced into the trachea with its tip proximal to the carina. Artificial ventilation with 100% oxygen was performed using a Servo ventilator 900C (Siemens-Elema, Solna, Sweden) with zero end-expiratory pressure, tidal volume (VT) of 12 ml/kg, respiratory frequency (f) of 30/min, and inspiratory to expiratory time

ratio (I/E) of 1:2. An infusion of 5% dextrose and 0.45% NaCl solution was continuously administered via the auricular vein as a maintenance fluid (7 ml/kg/h). Anesthesia was maintained with continuous infusion of pentobarbital (4 mg/kg/h) and fentanyl (120 μg/kg/h); muscle paralysis was achieved with pancuronium bromide (0.1 mg/kg/h).

A femoral artery and a femoral vein were cannulated with polyethylene catheters for arterial and central venous pressure monitoring and blood sampling. Arterial samples were analyzed for blood gases, pH, hemoglobin, and oxyhemoglobin saturation using conventional methods (ABL-330 and Osm-2 Hemoximeter, Radiometer, Copenhagen, Denmark). Arterial lactate was measured at baseline, after induction of respiratory failure, and at the end of experiments by an Eppendorf-Elen analyzer (Hamburg, Germany). A special indwelling catheter (Mikro-pO_2-Messkatheter, Licox, Germany) was inserted into the other femoral artery for continuous oxygen partial pressure monitoring (Licox, GMS, Kiel, Germany). Respiratory system compliance, airway pressures and airway resistances were measured on-line by a lung mechanics calculator 940 (Siemens-Elema, Sweden). This device allows lung mechanics calculations with consideration on compressible volume of the ventilator circuit and therefore reflects accurate data (13,14). End-tidal CO_2 (ET CO_2), CO_2 production (VCO_2), alveolar ventilation, and dead space ventilation were measured on-line by a CO_2 analyzer 930 (Siemens-Elema, Sweden). Core temperature was measured using an esophageal thermistor (Elektrolaboratoriet, Copenhagen, Denmark) and maintained at 37 ± 0.5°C by a heating blanket. Intravascular pressure monitoring was performed using Statham P23XL transducers (Spectramed, Oxnard, CA) zero-referenced to midthoracic level, and all variables, including electrocardiogram, were continuously traced on a Sirecust 1280 monitor (Siemens, USA) and recorded by a

Siredoc 220 recorder (Siemens, Erlangen, Germany).

Animals were studied supine. No additional drug treatment was attempted. At the end of the 3-h observation period, all animals were sacrificed with an overdose of pentobarbital and the lungs were prepared for histopathologic examination.

Treatment

Respiratory failure was induced by repeated lung lavage with warm saline (15,16) to achieve an arterial PO_2 below 100 mm Hg with the initial ventilator settings and positive end-expiratory pressure (PEEP) of 6 cm H_2O. The lung lavage model has been extensively used and is considered a reliable model to represent severe RDS with similar histologic and pathophysiologic changes (16-19).

After induction of respiratory failure, animals were divided randomly into three groups of six animals each. One group was treated with 18 ml/kg of intratracheal Perflubron (PFC group), another group received 18 ml/kg of intratracheal saline (saline group), and the last group received no treatment apart from continuous positive pressure ventilation (CPPV group). Treatment liquids were instilled in three consecutive doses of 6 ml/kg at 5-min intervals. At each instillation, animals were disconnected from the ventilator and treatment solution was administered directly into the endotracheal tube. During and after treatments, the ventilator settings were kept constant for all the groups as follows: volume-controlled ventilation, FiO_2 = 1.0, VT = 12 ml/kg, f = 30/min, I/E = 1:2 and PEEP = 6 cm H_2O.

Perflubron (perfluorooctyl bromide; Alliance Pharmaceutical Corp., San Diego, CA) is a PFC with a specific gravity of 1.918 g/cm^3 at 25°C, surface tension of 18.1 dynes/cm, vapor pressure of 3.6 mm Hg at 20°C and 10.5 mm Hg at 37°C, O_2 solubility of 53

ml/100 ml and CO_2 solubility of 210 ml/100 ml at 37°C and 1 atm pressure.

The experimental approach was to observe the animals for 3 h and compare them with respect to pulmonary gas exchange, lung mechanics, survival and postmortem lung histology. Measurements were determined at 30-min intervals, and continuous arterial PO_2 monitoring enabled observation of changes in PaO_2.

Histological examination

The thorax was opened through the anterior chest wall. The lungs were inflated with air to 30 cm H_2O for 5 min, and the pressure was then lowered to 10 cm H_2O; this was maintained while the lungs were perfused via the pulmonary artery with Davidson solution (20) under constant pressure. After fixation, the trachea was clamped and the lungs were removed en bloc and stored in 3.3% formalin. Representative sections of the lungs from several animals in each group were processed for histopathologic examination.

Statistical Analysis

All data are presented as mean ± standard deviation. Results were analyzed by multifactor analysis of variance. The statistical significance of the variables between pairs of groups was tested by the Newman-Keuls test. Measurements within groups were compared using the Wilcoxon signed-rank test. Values less than 0.05 were considered statistically significant.

RESULTS

The mean data before and after lung lavage were comparable in all groups. The PFC treatment was well tolerated in all animals. Three animals in the saline group did not

survive the experiment; one developed pneumothorax (at 100 min) and two died of cardiovascular collapse (at 80 and 100 min). In the CPPV group, one animal developed pneumothorax at 110 min.

Gas Exchange

The mean PaO_2, $PaCO_2$ and pH values are shown in figure 1. In the PFC group, PaO_2 increased significantly ($p<0.005$) from 67.2 ± 11.4 to 424.3 ± 14.0 mm Hg within 30 min and remained stable for 3 h; PaO_2 was 405.4 ± 53.2 mm Hg at the end of the experiments. In the CPPV group, PaO_2 stayed around 60 mm Hg during the observation period and differed significantly ($p<0.005$) from that in the PFC group. $PaCO_2$ in the PFC group remained remarkably stable, and mean values were comparable with the CPPV group for a period of 2 h. However, the mean $PaCO_2$ in the CPPV group subsequently increased from a postlavage value of 41.5 ± 4.2 to 58.2 ± 12.9 mm Hg at 3 h; $PaCO_2$ was not higher than the postlavage value in the PFC group at the end of the experiment. As expected, PaO_2 and $PaCO_2$ data showed severe deterioration in the saline group. There were decreases in pH in all groups, accompanied by metabolic acidosis. The changes in pH were less severe in the PFC group, dropping to 7.28 ± 0.05 at the end of the experiments; no bicarbonate was administered.

Alveolar ventilation and CO_2 gas-exchange parameters were well preserved for 3 h after treatment with Perflubron (table 1). VCO_2 was decreased towards the end of the study in the CPPV group and was severely impaired in the saline group. ET CO_2 monitoring revealed stable values in parallel to $PaCO_2$ both in the PFC and the CPPV groups; this was in contrast to the saline group, in which severe hypotension and low ET CO_2 levels were seen.

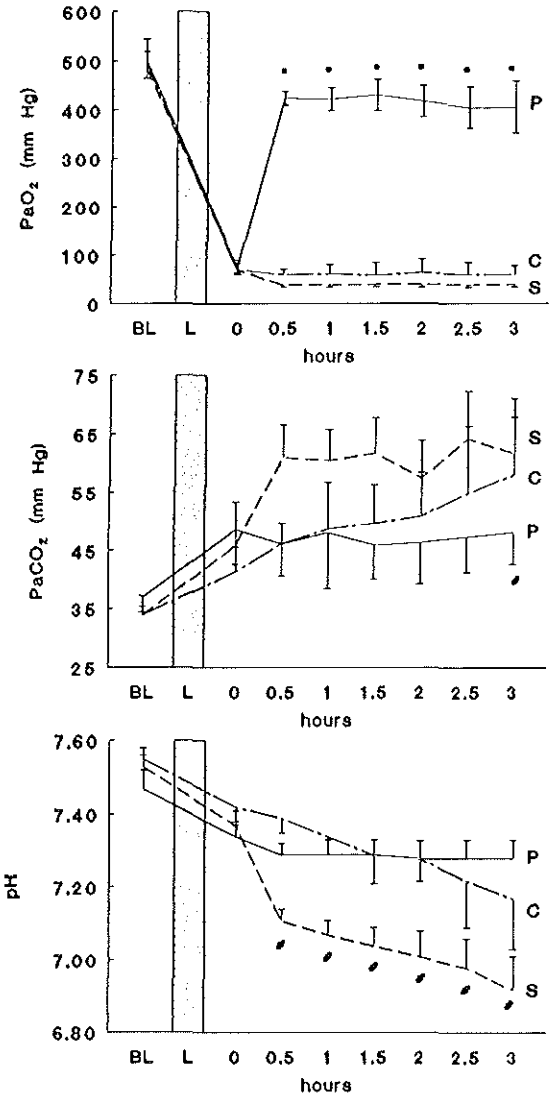

Figure 1. Arterial blood gases and pH in the treatment groups before lung lavage (BL), after lavage (0 point), and during the 3-h study period. Bar represents the lavage procedure (L). The mean values for PaO_2 are significantly different (*$p < 0.005$) in the PFC group (P) compared with the CPPV group (C) and the saline group (S) during 3-h observation period. The mean $PaCO_2$ at 3 h is significantly different (#$p < 0.05$) in the PFC group compared with other groups. The mean values for pH are significantly different (#$p < 0.05$) in the saline group compared with the other groups.

Lung Mechanics

The most remarkable observation in the PFC group was the significant ($p<0.05$) decrease in peak airway pressure (from the pretreatment value of 27.9 ± 2.5 to posttreatment 21.6 ± 1.6 cm H_2O) (figure 2) as well as in mean airway pressure (from 11.4 ± 0.4 to 10.4 ± 0.3 cm H_2O) (table 2). Thereafter, both airway pressures remained stable until the end of the study. There were increases in airway pressures in the CPPV and the saline groups during the course of the experiment.

Respiratory system compliance improved significantly with the PFC treatment and was maintained significantly higher ($p<0.05$) than the postlavage value throughout the experiment, in contrast to the CPPV group (figure 3). Inspiratory airway resistance decreased significantly only in the PFC group. Expiratory airway resistances were unchanged and comparable in all groups (table 2).

Cardiovascular Parameters

Animals tolerated large amounts of PFC instillation without significant changes in mean arterial pressure, heart rate and central venous pressure for 3 h (table 3). In the CPPV group, heart rate terminally decreased to about 75% of the baseline values, whereas in the saline group the same decreases were accompanied by severe impairment of the mean arterial pressure, most likely due to severe hypoxia. Metabolic acidosis was recorded at the end of the experiment in all groups, with the lowest arterial lactate levels in the PFC group (table 3).

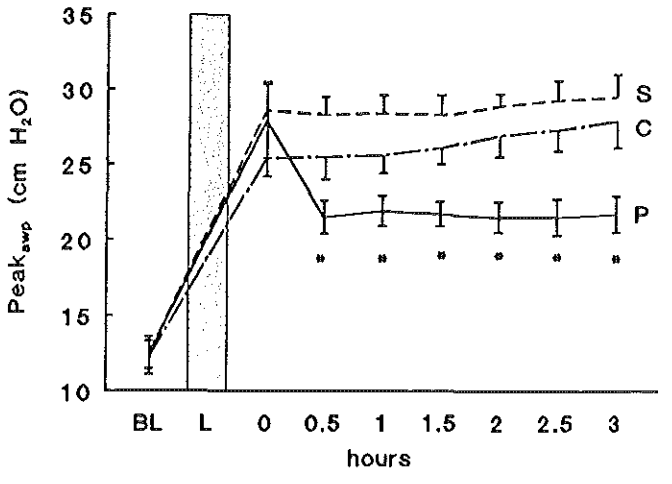

Figure 2. Peak airway pressures in the PFC group (P), saline group (S) and CPPV group (C) before lavage (BL), after lavage (0 point) and during the 3-h study period. The ventilator settings are kept the same in all groups before and after lung lavage, except that a PEEP of 6 cm H_2O is applied during lung lavage (L). In the PFC group, peak airway pressure differs significantly (*$p < 0.005$) from both the saline and CPPV groups during the 3-h observation period.

Table 1. Gas Exchange and ET CO_2

	VA (L/min)			Vco_2 (ml/min)			ET CO_2 (%)		
	PFC	Saline	CPPV	PFC	Saline	CPPV	PFC	Saline	CPPV
Pre	0.75±0.06	0.82±0.12	0.60±0.09	18.8±2.5	19.3±3.0	19.0±1.8	3.20±0.2	3.19±0.2	3.26±0.1
Post&	0.55±0.06	0.68±0.12	0.59±0.10	17.5±2.3	16.2±3.3	17.0±1.8	3.51±0.3	3.31±0.4	3.44±0.1
30	0.55±0.06	0.58±0.21	0.62±0.10	17.3±2.3	14.2±1.2	16.0±2.3	3.53±0.3	3.40±0.5	3.52±0.1
60	0.60±0.11	0.60±0.25	0.62±0.08	17.7±2.0#	13.7±1.5	15.7±2.7	3.51±0.3	3.26±0.4	3.48±0.1
90	0.60±0.11	0.60±0.20	0.60±0.09	17.8±1.9#	13.0±1.9	15.5±2.7	3.58±0.4	3.10±0.3	3.70±0.3
120*	0.60±0.11	0.60±0.27	0.58±0.08	18.0±1.7#	12.0±2.7	15.8±2.8	3.54±0.4	2.88±0.2	3.72±0.4
150*	0.57±0.05	0.60±0.27	0.58±0.08	18.3±1.8#	11.7±2.5	15.2±3.1	3.56±0.3	2.88±0.3	3.73±0.4
180*	0.57±0.05	0.60±0.27	0.56±0.09	18.5±1.4#	11.7±2.5	14.0±3.5	3.58±0.3	2.77±0.4	3.70±0.3

Definition of abbreviations: V_A = alveolar ventilation; Vco_2 = CO_2 production; ET CO_2 = end-tidal CO_2; Pre = prelavage; Post = postlavage; & = Values are minutes after lavage; * = Mean data from three animals in the saline group; # = $p < 0.05$ versus other groups.

Table 2. Lung Mechanics

	$Mean_{awp}$ (cm H_2O)			R_I (cm H_2O/L/sec)			R_E (cm H_2O/L/sec)		
	PFC	Saline	CPPV	PFC	Saline	CPPV	PFC	Saline	CPPV
Pre	3.4±0.3	3.6±0.3	3.4±0.2	19.3±4.2	19.0±6.5	16.3±3.5	98.5± 12.1	91.8± 13.1	74.2± 15.4
Post&	11.4±0.4	11.5±0.6	11.3±0.4	36.3±4.6	34.7±4.2	31.7±2.0	117.2± 0.8	117.3± 0.5	117.0± 0.0
30	10.2±0.2#¶	12.4±0.5¶	11.3±0.4	30.5±4.4¶	33.2±4.5	33.2±4.4	112.7± 8.0	117.2± 0.4	117.0± 0.0
60	10.3±0.2#¶	12.3±0.6¶	11.3±0.4	31.3±4.2¶	31.5±5.4	32.2±4.3	110.5± 10.5	117.0± 0.0	117.5± 0.6
90	10.2±0.3#¶	12.3±0.5¶	11.5±0.5	32.0±4.2¶	31.8±4.3	31.2±5.1	112.2± 5.9	117.2± 0.5	117.5± 0.6
120*	10.2±0.3#¶	12.4±0.6	11.7±0.7	31.8±4.2¶	27.3±5.9	29.8±4.7	113.7± 5.4	117.3±0.6	117.0± 0.7
150*	10.2±0.3#¶	12.3±0.5	11.7±0.6	32.0±4.7¶	27.0±7.0	29.0±3.9	110.2± 8.5	117.3±0.6	117.6± 0.9
180*	10.2±0.3#¶	12.3±0.6	11.8±0.7	31.5±3.9¶	25.3±8.4	28.6±2.9	113.0± 6.4	117.3±0.6	117.6± 0.6

Definition of abbreviations: $Mean_{awp}$=mean airway pressure; R_I= inspiratory airway resistance; R_E= expiratory airway resistance; Pre = prelavage; Post = postlavage.
& = Values are minutes after lavage; * = Mean data from three animals in the saline group; # = $p < 0.05$ versus other groups; ¶ = $p < 0.05$ versus pretreatment value (postlavage).

Table 3. Hemodynamics and Lactate

	MAP (mm Hg)			CVP (mm Hg)			HR (beats/min)			Lactate (mmol/L)		
	PFC	Saline	CPPV	PFC	Saline	CPPV	PFC	Saline	CPPV	PFC	Saline	CPPV
Pre	98±6	96±6	100±5	5±0.5	4±0.8	3±1.0	323±21	340±12	350±12	3.36±1.7	2.65±1.0	2.35±0.3
Post&	87±10	91±6	100±9	5±1.0	4±1.0	4±0.6	315±23	328±18	335±21	3.78±2.0	2.68±0.9	2.56±0.8
30	91±7	68±7#¶	101±10	5±0.8	4±0.4	4±0.5	303±26	303±20	318±18			
60	92±9	62±9#¶	100±10	5±0.9	4±0.6	4±1.0	300±27	298±20	293±25			
90	92±6	55±12#¶	97±12	5±1.0	4±0.8	4±0.6	295±23	282±13	255±33			
120*	95±9	53±10#	100±13	5±0.6	4±1.0	4±0.9	288±26	275±9	240±46			
150*	94±7	47±8#	94±9	5±0.6	5±0.6	4±0.5	290±26	265±17	249±36			
180*	91±7	44±12#	91±5	5±0.7	4±1.0	4±0.7	285±21	250±23	246±36	6.64±2.4¶	12.4±1.8#¶	7.89±2.5¶

Definition of abbreviations: MAP = mean arterial pressure; CVP = central venous pressure; HR = heart rate; Pre = prelavage; Post = postlavage.
& = Values are minutes after lavage; * = Mean data from three animals in the saline group; # = $p < 0.05$ versus other groups; ¶ = $p < 0.05$ versus pretreatment value (postlavage).

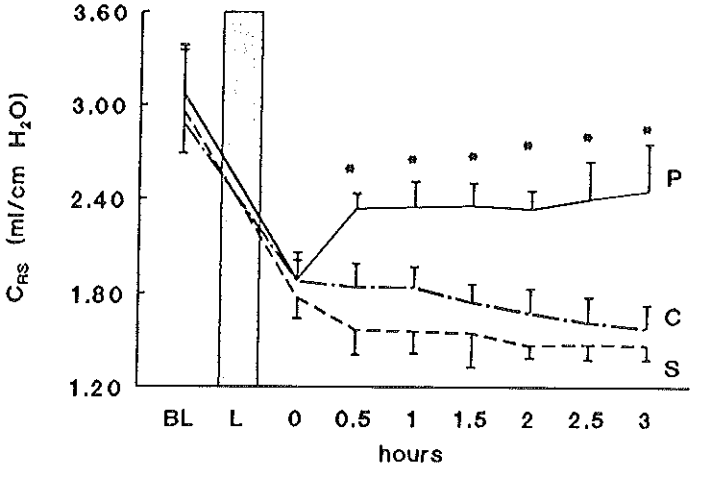

Figure 3. Changes in respiratory system compliance (C_{RS}) in the PFC group (P), saline group (S), and CPPV group (C) (BL = before lung lavage; L = lavage procedure). After treatment, respiratory system compliance is significantly (*$p < 0.005$) higher in the PFC group compared with the other groups throughout the study.

Lung Histology

Macroscopically, the lungs from animals of the PFC group and the saline group appeared to be well aerated; in the CPPV group some regions were observed to be atelectatic at end expiration. Microscopic examination of the lungs from several animals in each group did not reveal any significant morphologic abnormalities in the PFC or saline groups, but the lungs from animals of the CPPV group showed some degree of alveolar damage, involving distension and rupture of alveoli (figure 4).

DISCUSSION

This study demonstrated that intratracheal PFC administration combined with conventional mechanical ventilation can provide better pulmonary gas exchange at lower airway pressures for several hours compared to CPPV at identical ventilator settings in animals with this model of respiratory failure. High volumes of PFC were well tolerated without cardiovascular impairment; discernible treatment-related alveolar damage was not seen on histological analysis. The treatment volume of PFC in this experiment (18 ml/kg) corresponds to the functional residual capacity for healthy rabbits of identical body weight and PEEP level (unpublished observations).

The aim of ventilatory therapy in respiratory failure is to maintain adequate gas exchange in the lung by opening and stabilizing closed units with the least possible barotrauma and circulatory impairment. Alveoli in lungs with a damaged surfactant system have a great tendency to collapse during expiration, and therefore efficient modes of ventilation must overcome forces related to increased surface tension in the alveoli during both the inspiratory and expiratory phase of the ventilatory cycle. In clinical practice, this balance is not easily achieved because of the dependence of ventilatory volumes and airway pressures on ventilator settings. Therefore, numerous respiratory support techniques have been introduced and clinically investigated, aiming to define the optimal ventilator therapy to improve lung function and avoid high airway pressure (1-6). The latter is mostly considered the main risk factor for progressive deterioration in lung functions (21-23).

The improvement and maintenance of PaO_2 for several hours and the observed

decreases in airway pressures in this experiment imply the possible mechanisms of action of this technique "partial liquid ventilation" in an animal model of respiratory failure. The instantaneous and remarkable improvement of PaO_2 suggested that, when administered at volumes equal to FRC volume, PFC easily opens up atelectatic areas and prevents intermittent alveolar collapse in surfactant-depleted lungs at a PEEP level of 6 cm H_2O. We speculate that once the lung volume is increased and maintained by PFC liquid during the expiratory phase of respiratory cycle, continuous pulmonary gas exchange is facilitated and thus intrapulmonary shunt due to end-expiratory collapse of the alveoli is eliminated. This was confirmed by the well-sustained PaO_2 (above 400 mm Hg) for 3 h, as well as by the histological findings, which revealed almost no atelectatic areas at end expiration in the PFC-filled lungs. The alveoli filled with PFC can thus take up oxygen because oxygen dissolves easily in PFC. The inability to support oxygenation and CO_2 elimination in the saline group also revealed that the improved gas exchange in the PFC group was contributed by Perflubron itself and its high solubility for O_2 and CO_2.

On the other hand, replacing the high surface tension at the air/liquid interface by PFC eliminates the increased alveolar surface tension in the surfactant-deficient lung during partial liquid ventilation, so that lung inflation with the same volumes occurs at lower airway pressures. An interesting observation was that the peak airway pressures decreased in all animals as soon as 2 to 3 min after administration of 6 ml/kg Perflubron, remaining almost unchanged with subsequent doses. The data demonstrated an average of 23% decrease in peak airway pressures with a concomitant increase of 25% in respiratory system compliance following PFC administration. This finding supports the

previous study, which demonstrated that, in a similar surfactant-deficient model, peak airway pressure was already decreased after intratracheal administration of 3 ml/kg Perflubron (unpublished observations). From these results, one may speculate that a thin film of PFC liquid with a low surface tension is acting throughout almost the whole lung and thus reducing the elevated alveolar surface tension at the air/liquid interface. This action of PFC strongly suggests that during partial liquid ventilation the lungs behave almost as a fluid-filled lung in which the alveolar expansion with increasing lung volume occurs with very small increments of pressure until tissue elasticity becomes the major determinant of pressure changes in the mechanical behavior of the lung (24). This assumption is merely speculative, but from the experimental results it is difficult to find any other hypothesis for this behavior of lavaged lungs.

On the other hand, another factor to be considered in interpretation of lung mechanics during PFC application is the hydrostatic effect of PFC liquid. Because of the high density of PFC liquids, the dependent parts of the lung are likely to be exposed to additional hydrostatic pressures during PFC application techniques, and thus the alveolar pressure depends on both the airway pressure and the localization of the alveoli in the thorax. As suggested by Fuhrman and colleagues (12), however, the exact contribution of PFC to alveolar pressures is difficult to evaluate during mechanical ventilation with intratracheal PFC application and deserves further investigations.

Our findings regarding the improvements in lung mechanics (for example, decreases in airway pressures and increases in respiratory system compliance) in this animal model of respiratory failure are consistent with liquid ventilation studies in animals with RDS in which similar improvements in lung mechanics were observed on

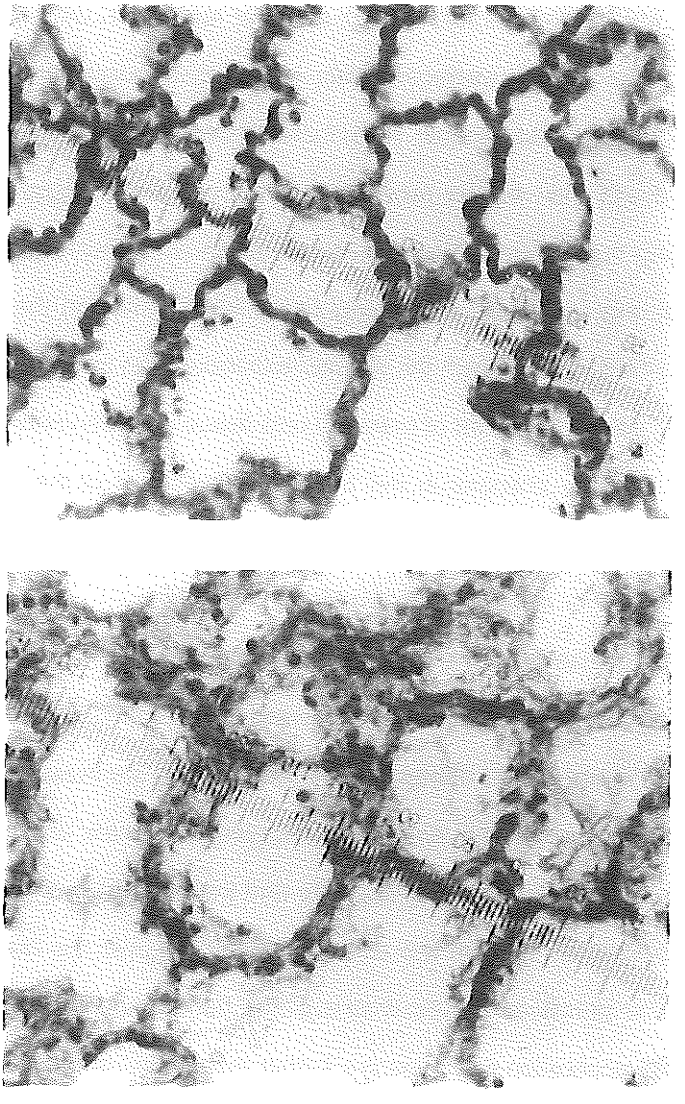

Figure 4. Lung sections from animals ventilated for 3 h after lung lavage in combination with intratracheal PFC administration (A) and without any treatment (CPPV) (B). The alveoli in the PFC group are well aerated and alveolar structure is intact, whereas the alveolar structure is irregular, with prominent exudate and hyaline membrane formation in the CPPV group. Hematoxylin and eosin stain. Original magnification: x250.

reconversion to conventional gas ventilation and were attributed to the remaining PFC in the lungs (8,10). Therefore, from all these results it can be speculated that the improvements in lung mechanics following intratracheal PFC administration should be a result of reduction of elevated alveolar surface tension at the air/liquid interface since this is the major determinant of elastic recoil pressure within 70% of total lung capacity (24).

The advantage of combining intratracheal PFC administration with conventional mechanical ventilation under the present experimental conditions is obvious and supports the importance of a low surface tension at the air/liquid interface if one compares the results with those from the CPPV group. Insufficient arterial oxygenation, high peak airway pressure, development of pneumothorax, and more prominent histological lung damage were seen at identical ventilator settings in surfactant-deficient and untreated lungs. Every effort to improve gas exchange with conventional volume-controlled ventilation would cause inevitable increases in airway pressures and would result in increased risk of pneumothorax. In our experiments, animals developed pneumothorax at relatively low peak airway pressures (30 to 33 cm H_2O) compared with clinical practice, which is presumably a species-related characteristic and accords with our experimental experience.

Considering the data, it can be suggested that the progress of functional lung impairment due to high peak airway pressures can be prevented or minimized by instillation of Perflubron into diseased lungs during mechanical support. This gives the diseased lung a chance to maintain its normal functions until the primary disorder is cured. This was confirmed by the histological findings that there did not appear to be

significant morphologic abnormalities in the alveolar structure of the PFC group. Numerous histological studies have also revealed that total liquid ventilation (using liquid ventilators and extracorporeal oxygenation) is less harmful to lung structures than conventional gas ventilation (25-27).

In previous studies with total liquid ventilation, high inspiratory airway pressures have been reported, with increases in pulmonary vascular resistance and decreases in cardiac output associated with high airway pressures (28-30). Our data are consistent with the paper of Fuhrman and colleagues, who demonstrated that, using the same technique of PFC and gas ventilation in healthy animals, adequate gas exchange could be maintained at airway pressures comparable to CPPV (12).

Although there were no significant changes in the cardiovascular parameters and arterial oxygenation was well maintained, elevated arterial lactate levels in the PFC group (although lower than in the other groups) were measured. Despite the well-maintained body temperature and the stable metabolic rate of the animals as evaluated by VCO_2, the elevated arterial lactate levels in the PFC group remain unexplained. The occurrence of lactic acidosis in the CPPV group and the saline group was not surprising since there were accompanying impaired aerobic metabolic activity, as reflected by the decreased VCO_2 values at 3 h in both groups (27 ± 14% and 34 ± 7% decreases from the baseline, respectively). A few investigations have demonstrated decreased cardiac output and accompanying acidosis during total liquid ventilation, attributed to the cardiopulmonary interactions as a result of filling the lung with heavy PFC liquid (28-30). A recent report by Curtis and colleagues demonstrated that hemodynamic compromise during liquid ventilation can be prevented or corrected by adequate intravascular fluid

volume (31); after intravascular fluid challenge initiated before liquid ventilation, oxygen consumption and lactate levels remained stable in healthy animals during 2 h of liquid ventilation.

Small amounts of PFC have been measured in blood and in some tissues after liquid ventilation in human neonates (32) and one may speculate about the possible contribution of decreased lactate metabolism in the liver caused by the PFC transported into the blood across the alveolar membrane. However, reports on the intravenous administration of Perflubron in humans have demonstrated no significant adverse effects (33). Moreover, in another study on healthy rabbits treated with intratracheal PFC, normal lactate levels were measured after 3 h of mechanical ventilation (unpublished observations). Further studies are required to clarify the mechanism of elevated lactate levels in the PFC-treated animals, although it is likely that this will be a reflection of cardiovascular dysfunction because mechanical ventilation with PEEP may alter cardiac output and distribution of blood flow to many organs by various mechanisms (34, 35).

Finally, this study provided indirect data related to the evaporative loss of the tested PFC and the alveolar oxygen level. Because PFC liquids are metabolically inert and eliminated mainly via the expired air, it is logical to assume that the evaporation of the PFC liquid would be an important determinant for the duration of the favorable effects of PFC with this new technique. Perflubron has an extremely low vapor pressure (10.5 mm Hg at 37°C and 1 atm pressure) compared with currently available PFC liquids. Thus, because of its low rate of elimination by evaporation, improvements in arterial blood gases and lung mechanics were maintained for 3 h in this experiment, without necessitating Perflubron replacement. On the other hand, the presence of saturated

Perflubron vapor would decrease the maximal obtainable alveolar oxygen pressure by 10.5 mm Hg, much less than with other higher vapor pressure PFC. For long-term application, the vapor pressure of PFC liquids appears to be of great importance in this type of ventilatory support from a cost perspective because of the evaporative loss and for the level of alveolar oxygen pressure in severe cases.

In conclusion, this study demonstrates that (1) adult animals with acute respiratory failure can be supported with intratracheal Perflubron administration combined with conventional mechanical ventilation to improve pulmonary gas exchange and lung mechanics; (2) at identical ventilator settings, this type of ventilatory support proved to be significantly more effective than conventional gas ventilation by ensuring improved gas exchange at relatively low inflation pressures; (3) a Perflubron volume approximately equal to FRC volume can be tolerated for several hours without impairing hemodynamic stability; (4) the extremely low vapor pressure of Perflubron makes it an ideal PFC liquid for this new type of ventilatory support for long-term application.

A total liquid ventilation technique involves administration of PFC liquid into the lungs in FRC volume and mechanical support of the lungs by extracorporeally oxygenated PFC liquid using a modified liquid ventilator. In contrast to the complexity of the total liquid ventilation technique, conventional gas ventilation using conventional ventilatory settings was used in these experiments. When the advantages of this new technique over total liquid ventilation (36) are taken into consideration, all these favorable effects emphasize the potential for clinical application of intratracheal PFC administration in combination with mechanical ventilation as an alternative treatment modality of RDS.

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CHAPTER 4

INTRATRACHEAL PERFLUOROCARBON ADMINISTRATION AS AN AID IN THE VENTILATORY MANAGEMENT OF RESPIRATORY DISTRESS SYNDROME

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INTRATRACHEAL PERFLUOROCARBON ADMINISTRATION AS AN AID IN THE VENTILATORY MANAGEMENT OF RESPIRATORY DISTRESS SYNDROME

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ABSTRACT

Background: Respiratory distress syndrome carries a high morbidity and mortality when treated with mechanical ventilation with positive end-expiratory pressure. Perfluorocarbon liquids are employed in liquid ventilation due to low surface tension and high gas solubility. To assess whether intratracheal administration of perfluorocarbon, perflubron, in combination with conventional mechanical ventilation could be of therapeutic benefit in respiratory distress syndrome, the authors tested the effects of different doses of intratracheal perflubron administration on gas exchange and lung mechanics in adult animals with respiratory failure during a 6-h observation period.

Methods: Respiratory failure was induced in 30 rabbits by saline lung lavage (arterial oxygen tension < 100 mmHg at 100% oxygen with the following ventilator settings: tidal volume, 12 $ml \cdot kg^{-1}$; respiratory frequency, 30 per minute;

inspiratory/expiratory ratio, 1:2; and positive end-expiratory pressure of 6 cm H_2O). Twenty-four rabbits were treated with different perfluorocarbon doses (3, 6, 9, and 12 $ml \cdot kg^{-1}$) and the remaining six served as controls while mechanical ventilation was continued with the aforementioned settings. Additionally, in ten healthy rabbits who were used as healthy controls, the lungs were mechanically ventilated either alone or in combination with intratracheal perfluorocarbon administration (3 $ml \cdot kg^{-1}$) for 6 h.

Results: In all treatment groups, arterial oxygen pressure increased significantly ($P < 0.0001$) in a dose-related fashion (193 ± 40, 320 ± 70, 353 ± 125 and 410 ± 45 mmHg at 15 min), and peak airway pressures decreased significantly (range, 18-23%; $P < 0.0001$) from pretreatment values. These findings were in contrast to those for control group. The improvements were time-dependent in all the four tested perfluorocarbon doses. However, the improvements in pulmonary parameters could be extended to 6 h only in groups treated with 9 $ml \cdot kg^{-1}$ and 12 $ml \cdot kg^{-1}$ perflubron. At the end of the 6-h period, the data for these two groups showed significantly higher arterial oxygen pressure (230 ± 84 and 197 ± 130 mmHg, respectively; $P < 0.05$) and lower inflation pressures then the pretreatment data for these groups and the data for the control group at 6 h. There were no clinically significant changes in pulmonary parameters in healthy animals due to either mechanical ventilation alone or mechanical ventilation in combination with intratracheal perfluorocarbon administration for 6 h.

Conclusions: The results of this study imply that there is no association between the lung mechanics and gas exchange parameters for mechanical ventilation in combination with intratracheal perfluorocarbon administration. The data suggest that this type of perfluorocarbon administration with conventional mechanical ventilation offers a

simple, alternative treatment of respiratory distress syndrome. With this technique, adequate pulmonary gas exchange can be maintained at relatively low airway pressures with high perfluorocarbon doses for several hours.

INTRODUCTION

Since the first successful experiment of Clark and Gollan,[1] the efficacy of perfluorocarbon liquids have been studied in liquid breathing techniques. Animal studies have shown perfluorocarbon liquid ventilation to be effective in achieving adequate pulmonary gas exchange in surfactant-deficient lungs by reducing surface tension at the alveolar air-liquid interface [2-4]. Improvements in lung functions have been reported following successful reconversion to gas breathing in diseased lungs.[3-6]

Despite the advances in liquid ventilation techniques, the complexity and the extra technical requirements (e.g., modified liquid ventilator, external oxygenator) for liquid ventilation have discouraged the development of this type of respiratory support in clinical practice. Greenspan et al.[7] reported the first human trial of liquid ventilation in premature infants with respiratory distress syndrome (RDS). The observed improvements in gas exchange and lung mechanics after only a short duration of liquid ventilation reaffirmed the results of animal experiments and led us to investigate a simpler way of using perfluorocarbon. Using a combination of conventional mechanical ventilation and intratracheal perfluorocarbon (perflubron, Alliance Pharmaceutical, San Diego, CA) administration at low doses, we recently demonstrated that oxygenation can, in the short-term, be improved in a dose-dependent manner at reduced airway pressures in adult

animals with acute respiratory failure [6]. This study was developed to assess the efficacy of this method of perfluorocarbon administration as an aid in the ventilatory management of RDS. This study was designed to investigate the effects of this technique with different perfluorocarbon doses on pulmonary gas exchange and respiratory mechanics in adult animals with induced RDS over a 6-hour observation period.

METHODS AND MATERIALS

This study was approved by the Animal Committee of Erasmus University Rotterdam.

Animal Preparation :

Adult New Zealand rabbits (n = 40) weighing 2.8 ± 0.3 kg were anesthetized with intravenous pentobarbital sodium (50 $mg \cdot kg^{-1}$) via an auricular vein and then placed in a supine position. An endotracheal tube (ID 3.5 mm) was inserted via tracheostomy, after which mechanical ventilation with a Servo Ventilator 900C (Siemens-Elema, Sweden) was initiated with 100% oxygen, zero end-expiratory pressure, tidal volume (VT) of 12 $ml \cdot kg^{-1}$, respiratory frequency of 30 per min and inspiratory/expiratory ratio of 1:2. An infusion of 5% dextrose/0.45% NaCl solution was administered continuously (7.5 $ml \cdot kg^{-1} \cdot h^{-1}$) via the auricular vein as a maintenance fluid. Anesthesia was maintained with continuous infusion of pentobarbital (4 $mg \cdot kg^{-1} \cdot h^{-1}$) and fentanyl (120 $\mu g \cdot kg^{-1} \cdot h^{-1}$); pancuronium bromide was administered to induced muscle paralysis (0.1 $mg \cdot kg^{-1} \cdot h^{-1}$).

A femoral artery and a femoral vein were cannulated with polyethylene catheters

for arterial and central venous pressure monitoring and blood sampling. Arterial samples were analyzed for blood gases, pH, and hemoglobin by conventional methods (ABL-330 and Osm-2 Hemoximeter, Radiometer, Copenhagen, Denmark). Arterial lactate was measured with an Eppendorf-Elan Analyzer (Hamburg, Germany) at baseline, after induction of RDS, and at the end of the 6-h observation period, using the Sigma Diagnostics Lactate Enzymatic Determination procedure (Lactate kit 735-10, Sigma, St. Louis, MO). End-tidal carbon dioxide, carbon dioxide production, and alveolar dead space were measured on-line with a carbon dioxide Analyzer 930 (Siemens, Sweden). Lung mechanics (airway pressures and respiratory compliance) were measured on-line with a Lung Mechanics Calculator 940 (Siemens, Sweden), which has been demonstrated to be a reliable and accurate calculator.[9] Core temperature was maintained at 37 ± 1•C with a heating blanket and monitored with an esophageal thermistor (Elektrolaboratoriet, Copenhagen, Denmark). Intravascular pressures were monitored with Statham P23XL transducers (Spectramed, Oxnard, CA), and all parameters, including electrocardiographic readings, were traced with a Sirecust 1280 monitor (Siemens, Danvers, MA) and recorded with a Siredoc 220 recorder (Siemens, Germany).

Experimental Protocol:

In 30 rabbits, acute respiratory failure was induced by lung lavage with 30 $ml \cdot kg^{-1}$ warm saline, [10,11] repeated as often as necessary to achieve an arterial oxygen pressure less than 100 mmHg at the following ventilator settings: volume controlled ventilation; inspired oxygen fraction, 1; VT, 12 $ml \cdot kg^{-1}$; respiratory frequency, 30 per min; inspiratory/expiratory ratio, 1:2; and positive end-expiratory pressure (PEEP), 6 cmH_2O.

The perfluorocarbon administered in this experiment was perflubron (perfluorooctyl

bromide; Alliance Pharmaceutical, San Diego, CA), a perfluorocarbon with a specific gravity of 1.918 $g \cdot cm^{-3}$ at 25°C, a surface tension of 18.1 dynes$\cdot cm^{-1}$, vapor pressure of 10.5 mmHg at 37°C, oxygen solubility of 53 $ml \cdot 100\ ml^{-1}$, and carbon dioxide solubility of 210 $ml \cdot 100\ ml^{-1}$ at 37°C and 1 atmosphere pressure.

Animals were divided randomly into five groups of six animals, and four groups were treated intratracheally with a different dose of perflubron: group 1, 3 $ml \cdot kg^{-1}$; group 2, 6 $ml \cdot kg^{-1}$; group 3, 9 $ml \cdot kg^{-1}$; and group 4, 12 $ml \cdot kg^{-1}$. In the fifth group, which was used as a control group (group C), no perflubron was used, and the animals' lungs were ventilated mechanically with gas. In the treated groups, perflubron was administered directly into the endotracheal tube in incremental volumes (at 5 min intervals) not to exceed 15 ml at one instillation. After each administration, the ventilator was immediately reconnected. The ventilator settings were kept constant as noted above, and mechanical ventilation was maintained for 6 h.

During the 6-h observation period, arterial blood gases, pH, and hemoglobin were determined initially at 15 min, and at 30 min intervals thereafter. Hemodynamic parameters (arterial pressure, central venous pressure, heart rate), lung mechanics, and carbon dioxide gas exchange parameters were recorded at the same time points. No additional drug treatment was attempted.

To determine any adverse effects of perflubron on the tested variables that might be obscured by the RDS state, a group of six healthy rabbits was given 3 $ml \cdot kg^{-1}$ of perflubron intratracheally following standard animal preparation. Before perflubron administration, PEEP was set to 2 cmH_2O, which was observed to be the minimal required PEEP to prevent the bulk movement of perflubron along the airways in each

respiratory cycle[6]. Measurements at this point were considered baseline. The animals' lungs were ventilated for 6 h with volume-controlled ventilation: inspired oxygen fraction, 1; VT = 12 ml•kg^{-1}; respiratory frequency, 30 per min; inspiratory/expiratory ratio, 1:2; and PEEP, 2 cmH_2O.

In another group of four healthy rabbits, no perflubron was administered, but the animals' lungs were ventilated mechanically for 6 h to test the effects of time alone on this preparation. The same ventilator settings used in the first healthy group were used in this group.

Arterial blood gases, pH determinations, and respiratory mechanics recordings were made at the same time points as in lung-lavaged animals. All animals were killed with an overdose of pentobarbital at the end of the 6-h observation period.

Statistical Analysis:

Results were analyzed by analysis of variance (ANOVA) for repeated measurements, using a maximum likelihood technique (Program 5V of BMDP package, BDMP Statistical Software, Los Angeles, CA), in which the dependent variable was modelled as a linear function of dose, time, time squared, and the interactions between dose and time squared. The statistical significance between all pairs of groups also was tested at separate points in time with the Student-Newman-Keuls test. Statistical analyses within each group were made by the Student's t-test. All data are presented as mean ± SD, unless otherwise stated. A P value of less than 0.05 was considered statistically significant.

RESULTS

Lung-lavaged Animals

In all groups of lung-lavaged animals, the measured and calculated variables were comparable before and after lung lavage. The saline lavage model used in this study represents a condition similar to RDS, with similar histological changes and pathophysiologic features, and therefore is considered as a reliable model.[11-13]

Gas Exchange:

All animals in groups 3 and 4 survived for the duration of the experiments. Two animals in group C developed pneumothorax after 3 h (at 200 and 310 min), five animals in group 1 developed pneumothorax after 3.5 h (at 240, 250, 270, 290, and 340 min), and three animals in group 2 developed pneumothorax after 5 h (at 305, 340, and 345 min). Suspicion of pneumothorax was based on observed acute increases in airway pressures and/or decreases in blood pressure in the animals. Suspicions were confirmed by direct inspection of the animals and/or aspiration of free air in the thorax through the diaphragm after an abdominal incision was made. In all the animals who underwent abdominal incision, pneumothorax was diagnosed, no treatment was applied, and measurements were discontinued in those animals. Therefore, data analyses are based on the remaining survivors.

Figure 1 depicts the response of arterial oxygen pressure to different doses of perflubron over the course of the experiments. After lung lavage (3 to 4 times), mean arterial oxygen tension (PaO_2) values of the groups were between 67 and 78 mmHg with PEEP of 6 cmH_2O before treatment. Compared to the control group, PaO_2 values

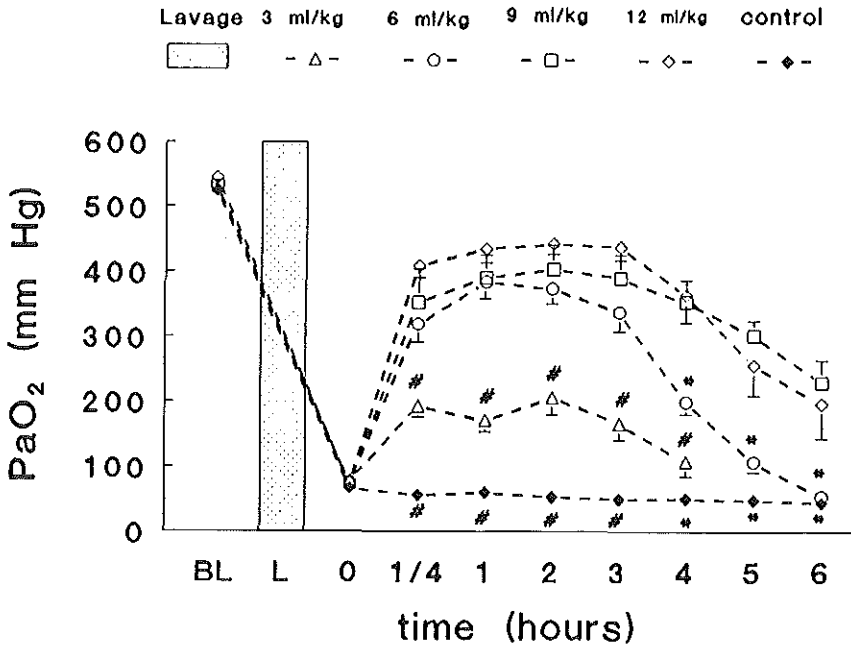

Figure 1. Arterial oxygen pressure (mean ± SEM) before lavage (BL), after lavage (0 point), and in response to treatment with perflubron at different doses. The bar represents the lavage procedure (L). Arterial oxygen tension increased significantly at each administration dose in conjunction with a decrease in alveolo-arterial oxygen gradient ($A\text{-}aDo_2$), reflecting an improvement of lung function, particularly a decrease in intrapulmonary shunt. The data for 3 $ml \cdot kg^{-1}$ group is not shown after 4 h because there were too few survivors. * = significantly different ($P < 0.0001$) from 9 and 12 $ml \cdot kg^{-1}$ groups; # = significantly different ($P < 0.0001$) from the other groups.

increased significantly ($P < 0.0001$) in all treatment groups in a dose-related manner with perflubron treatment. PaO_2 values were significantly lower ($P < 0.0001$) in group 1 than in the other treatment groups until 3.5 h; thereafter, PaO_2 values in groups 1 and 2 were significantly less ($P < 0.0001$) than in groups 3 and 4 at all time points. The mean PaO_2 values at 6 h remained significantly higher ($P < .05$) in groups 3 and 4 (230 ± 84 and 197 ± 130 mmHg, respectively) compared to the pretreatment values of these groups

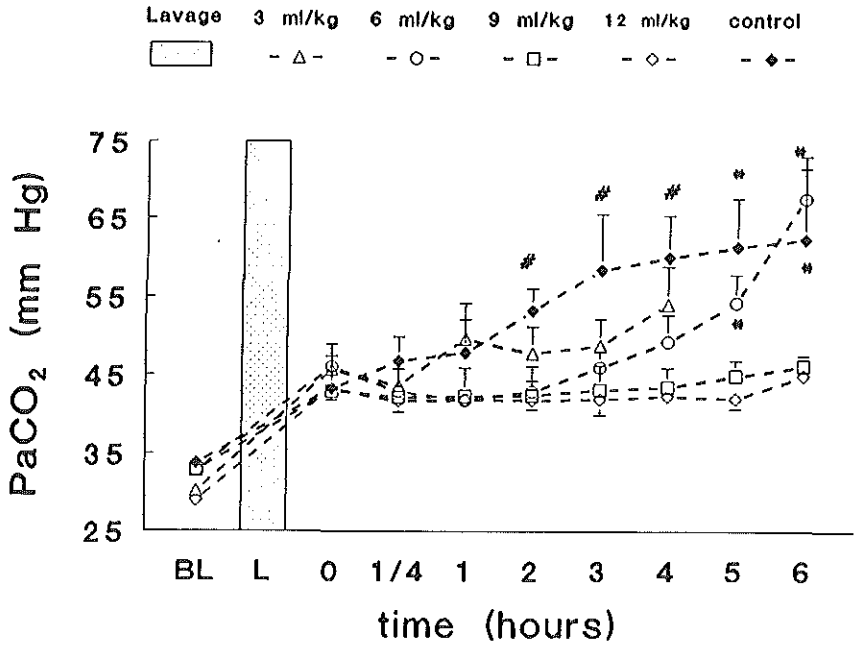

Figure 2. Arterial carbon dioxide pressure (mean ± SEM) before lavage (BL), after lavage (0 point), and after treatment with perflubron. Arterial carbon dioxide tension levels were more stable than arterial oxygen tension levels for 6 h in the groups treated with high doses (9 ml•kg^{-1} and 12 ml•kg^{-1}). Individual arterial carbon dioxide values in the 3 ml•kg^{-1} group were higher than those in the other groups after 1 h. The data for the 3 ml•kg^{-1} group is not shown after 4 h because there were too few survivors. * = significantly different ($P < 0.005$) from 9 and 12 ml•kg^{-1} groups; # = significantly different ($P < 0.005$) from 6, 9 and 12 ml•kg^{-1} groups. L = lavage procedure.

and the final readings of the control group. In the control group, PaO_2 gradually decreased to 45 ± 11 mmHg at 6 h.

Mechanical ventilation with tidal volume of 12 ml•kg^{-1} induced hypocapnia in all groups, but lung lavage caused significant increases ($P < 0.001$) in $PaCO_2$ and significant reductions ($P < 0.001$) in pH in all groups. After perflubron treatment, only in groups 3 and 4 were arterial PCO_2 values maintained at less than 45 mmHg throughout the study. These values were significantly different ($P < 0.005$) from those found in

groups C, 1, and 2 at 6 h (fig.2). There were significant decreases ($P < 0.05$) in pH in all groups toward the end of the observation period (mean pH value was slightly lower in group 1 before and after lung lavage compared to other groups; table 1). Despite the well maintained arterial carbon dioxide tensions in groups 3 and 4, these groups experienced metabolic acidosis. Respiratory acidosis accompanied metabolic acidosis in groups C, 1, and 2.

Compared to the control group, perflubron instillation decreased the alveolar dead space to VT ratio significantly ($P < 0.05$) in the treatment groups. In group 4, the decrease in the ratio of alveolar dead space to VT from the pretreatment level was insignificant (table 2). The lower the perflubron dose, the earlier the alveolar dead space to VT ratio started to increase.

Respiratory Mechanics:

After perflubron instillation, peak airway pressure ($Peak_{awp}$) needed to inflate the lungs with a VT of 12 $ml \cdot kg^{-1}$ decreased significantly ($P < 0.0001$), almost to the same extent (range, 17-21%) in all treatment groups at 15 min (fig.3). On the other hand, airway pressures needed to inflate the lungs increased significantly ($P < 0.001$) over time in the control group. To reflect the actual intrathoracic pressures, the data for end-inspiratory pause airway pressure ($Pause_{awp}$) and mean airway pressure ($Mean_{awp}$) are presented in table 2. The airway pressures ($Peak_{awp}$, $Pause_{awp}$, and $Mean_{awp}$) were significantly lower ($P < 0.0001$) in the treatment groups than in the control group at 6 h, and in groups 3 and 4 compared to groups 1 and 2 from 3.5 h. Only groups 3 and 4 exhibited airway pressures significantly lower ($P < 0.05$) than the pretreatment values, even at 6 h.

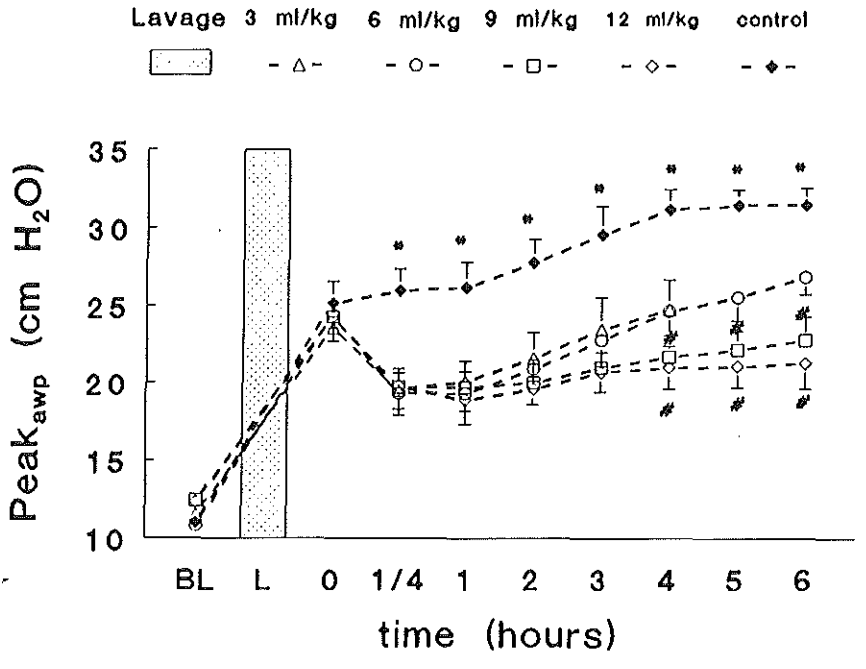

Figure 3. Changes in peak airway pressure ($Peak_{awp}$; mean ± SEM) in response to perflubron treatment. Positive end-expiratory pressure of 6 cm H_2O is applied during lavage procedure (L), and ventilator settings are kept constant in all groups before and after treatment. Although perflubron administration provided on average a 20% decrease in peak airway pressure in all groups, the linear correlation between peak airway pressure and the function of time is different for each group, favoring 12 ml•kg^{-1} treated group to exhibit lower values at all time points. The data for 3 ml•kg^{-1} group is not shown after 4 h because there were too few survivors. * = significantly different ($p<0.0001$) from other groups; # = significantly different ($p<0.0001$) from 3 and 6 ml•kg^{-1} and control groups. BL = before lavage.

Respiratory system compliance (CRS; ml•cmH_2O^{-1}•kg^{-1}) increased significantly ($P < 0.0001$) in all treatment groups after treatment with perflubron, but in the control group, this value decreased (fig.4). For the first 2 h, there were no statistically significant differences in CRS in groups 3 and 4 compared to CRS before lung lavage. Mean values of CRS in groups 3 and 4 remained significantly higher ($P < 0.0001$) than those in groups 1 and 2 after 3 h. At the end of the observation period, CRS was still significantly

TABLE 1. Hemodynamics, pH and metabolic rate data of control and treatment groups

	Group	Before Lavage	After Lavage	15 min	1 h	2 h	3 h	4 h	5 h¶	6 h¶
MAP	C	93±15	87±18	88±18	86±11	87±13	82±13	81±16	84±17	86±18
	G1	95±9	95±6	89±10	87±3	91±4	90±8	90±14	-	-
	G2	93±8	89±10	88±10	86±12	82±5	84±9	88±10	84±7	84±3
	G3	93±11	83±12	92±11	90±14	89±5	94±9	95±8	96±9	97±6
	G4	90±17	97±21	89±15	90±11	86±8	88±10	88±10	86±14	85±16
HR	C	308±17	297±16	304±13	299±16	285±21	274±15*	261±15*	268±16*	263±14*
	G1	315±27	308±40	298±37	280±30	278±31	268±18*	279±13	-	-
	G2	333±36	328±6	318±15	300±19*	270±16*	278±16*	270±21*	258±26*	255±30*
	G3	315±21	318±11	313±18	289±13*	285±19*	285±13*	280±16*	285±23*	288±24*
	G4	323±13	310±10	313±15	298±22	283±31	288±15*	283±15*	288±20*	268±22*
pH	C	7.51±.04	7.37±.04	7.36±.04	7.33±.02*	7.29±.02*	7.24±.05*#	7.21±.03*#	7.19±.06*#	7.19±.04*&
	G1	7.49±.04	7.33±.05	7.33±.05	7.30±.05#	7.31±.06	7.30±.06	7.26±.10	-	-
	G2	7.64±.06	7.38±.03	7.41±.03	7.40±.03	7.37±.03	7.35±.04	7.32±.03*	7.29±.02*	7.23±.05*
	G3	7.53±.05	7.40±.08	7.39±.10	7.38±.08	7.36±.08	7.33±.08	7.31±.07	7.29±.06*	7.29±.04*
	G4	7.61±.05	7.39±.03	7.42±.02	7.40±.04	7.37±.04	7.35±.04	7.34±.03*	7.32±.04*	7.30±.03*
Vco_2	C	7.7±1.2	6.6±1.6	7.2±1.2	6.9±0.8	7.1±1.1	6.7±0.5	6.6±1.2	6.4±1.2	6.8±0.9
	G1	6.6±1.0	5.7±0.7	6.3±0.9	6.2±1.1	6.3±1.0	6.0±0.8	6.1±0.9	-	-
	G2	7.4±0.7	5.7±0.7	7.3±0.8	6.7±0.6	6.2±0.4	6.0±0.3	5.9±0.5	5.6±0.9	5.6±1.3
	G3	6.7±1.1	5.4±0.9	6.8±1.0	6.6±0.4	6.4±0.4	6.1±0.3	6.0±0.3	6.1±0.4	5.8±0.6
	G4	7.4±0.9	6.5±0.7	7.2±0.6	7.1±0.4	6.8±0.7	6.8±0.4	6.6±0.4	6.5±0.5	6.5±0.5

Data are mean ± SD.
MAP=mean arterial pressure (mm Hg); HR=heart rate (beats per min); Vco_2=CO_2 production ($ml \cdot kg^{-1} \cdot min^{-1}$); C = control; G1 = 3 $ml \cdot kg^{-1}$; G2 = 6 $ml \cdot kg^{-1}$; G3 = 9 $ml \cdot kg^{-1}$; G4 = 12 $ml \cdot kg^{-1}$.
¶Data for Group 1 is not depicted because of too few survivors.
*Statistically different ($p<0.05$) from pretreatment value (after lavage).
#Statistically different ($p<0.05$) from G2, G3 and G4.
&Statistically different ($p<0.01$) from G3 and G4.

TABLE 2. Ventilatory parameters of control and treatment groups

	Group	Before Lavage	After Lavage	15 min	1 h	2 h	3 h	4 h	5 h¶	6 h¶
$Pause_{awp}$	C	11.0±2.0	25.2±1.4	26.0±1.4§	26.2±1.6§	27.8±1.5*§	29.6±1.8*§	31.2±1.3*§	31.5±1.0*§	31.5±1.1*§
	G1	11.5±1.7	23.6±0.7	19.5±1.4*	20.1±1.3*	21.6±1.7*	23.5±2.1	24.8±1.9&	-	-
	G2	10.8±1.7	24.4±0.8	19.3±1.0*	19.3±1.1*	20.9±0.8*	22.8±1.7	24.7±1.6&	25.6±1.5&	26.9±1.1*&
	G3	12.4±1.1	24.3±0.5	19.7±0.9*	19.7±1.0*	20.0±1.5*	21.0±1.0*	21.7±0.7*	22.2±1.2*	22.8±1.5*
	G4	12.3±1.2	24.3±1.6	19.8±1.9*	18.9±1.6*	19.6±1.0*	20.7±1.3*	21.0±1.4*	21.1±1.4*	21.3±1.7*
$Mean_{awp}$	C	3.4±0.5	11.9±0.4	12.1±0.4§	12.2±0.5§	12.6±0.5*§	12.9±0.4*§	13.2±0.4*§	13.3±0.4*§	13.4±0.4*§
	G1	3.8±0.7	11.3±0.3	10.2±0.4*	10.3±0.5*	10.7±0.5*	11.0±0.6&	11.3±0.7&	-	-
	G2	3.6±0.5	11.4±0.4	10.0±0.3*	10.0±0.2*	10.3±0.3*	10.9±0.4*	11.2±0.4&	11.4±0.5&	11.6±0.3&
	G3	4.1±0.3	11.3±0.2	10.0±0.3*	9.8±0.3*	9.9±0.4*	10.2±0.4*	10.5±0.3*	10.5±0.4*	10.6±0.4*
	G4	4.0±0.2	11.4±0.7	10.0±0.5*	9.7±0.4*	9.9±0.4*	10.2±0.4*	10.3±0.5*	10.3±0.4*	10.3±0.5*
V_D/V_T	C	0.25±0.02	0.39±0.05	0.39±0.06	0.39±0.05	0.41±0.05	0.41±0.06	0.44±0.03	0.42±0.07	0.38±0.06
	G1	0.28±0.02	0.44±0.04	0.38±0.04*	0.40±0.04	0.42±0.05	0.43±0.03	0.46±0.05	-	-
	G2	0.27±0.02	0.46±0.04	0.40±0.03*	0.39±0.04*	0.43±0.03	0.45±0.03#	0.49±0.04	0.49±0.04	0.51±0.03#
	G3	0.28±0.02	0.45±0.04	0.40±0.03*	0.39±0.04*	0.40±0.04*	0.41±0.05	0.43±0.03	0.42±0.02	0.43±0.02#
	G4	0.27±0.02	0.41±0.07	0.37±0.02	0.36±0.05	0.36±0.04	0.36±0.05	0.36±0.04§	0.35±0.03§	0.36±0.04

Data are mean ± SD.
$Pause_{awp}$=end-inspiratory airway pressure (cm H_2O); $Mean_{awp}$=mean airway pressure (cm H_2O); V_D/V_T=dead space to tidal volume ratio; C = control; G1 = 3 ml•kg^{-1}; G2 = 6 ml•kg^{-1}; G3 = 9 ml•kg^{-1}; G4 = 12 ml•kg^{-1}.
¶ Data for group 1 is not depicted because of too few survivors.
* Statistically different ($p<0.05$) from pretreatment value (AL).
& Statistically different ($p<0.0001$) from G3 and G4.
# Statistically different ($p<0.001$) from G4.
§ Statistically different ($p<0.0001$) from other groups.

higher ($P < 0.05$) in group 4 only (2.29 ± 0.20 ml•cmH_2O^{-1}•kg^{-1}) compared with this group's pretreatment value (1.90 ± 0.16 ml•cmH_2O^{-1}•kg^{-1}).

Hemodynamics:

Mean arterial pressure and central venous pressure (5 ± 1 mmHg at pretreatment) remained stable in all groups throughout the observation period. There were significant changes ($P < 0.05$) in heart rate in all groups towards the end of the study (table 1).

Although carbon dioxide production decreased slightly in all groups, at the end of the study (table 2), arterial lactate levels had increased significantly ($P < 0.0001$) in the control group (from 2.6 ± 0.7 to 8.2 ± 1.5 $mM \cdot L^{-1}$), group 1 (from 2.8 ± 0.7 to 9.4 ± 2.5 $mM \cdot L^{-1}$), and group 4 (from 3.0 ± 0.9 to 8.0 ± 1.5 $mM \cdot L^{-1}$). These levels had increased insignificantly in groups 2 and 3 (from 3.0 ± 1.1 to 5.0 ± 2.5 $mM \cdot L^{-1}$ and from 2.9 ± 0.9 to 4.2 ± 1.5 $mM \cdot L^{-1}$, respectively) at 6 h from pretreatment levels.

Healthy Animals

All animals survived for the duration of the experiment, and the baseline data were comparable in both groups. Animals tolerated intratracheal perflubron administration well. There were no harmful effects because of mechanical ventilation alone for 6 h, nor because of mechanical ventilation in combination with intratracheal perflubron (3 $ml \cdot kg^{-1}$) administration on measured parameters in healthy animals during the 6 h study period.

There was no change in arterial oxygenation following perflubron administration for 2 h compared to baseline level: mean PaO_2 remained higher than 500 mmHg; pulmonary gas exchange parameters were comparable, and there were no clinically significant changes in both groups during the 6 h observation period (table 3).

The only significant difference between the two groups was in respiratory mechanics parameters (table 3). When compared to baseline level, airway pressures ($Peak_{awp}$, $Pause_{awp}$, and $Mean_{awp}$) were significantly higher ($P < 0.05$) in the perflubron-treated group for 4 h, but decreased to baseline levels thereafter. However, $Peak_{awp}$ and $Pause_{awp}$ did not differ significantly between the two groups throughout the study.

TABLE 3. Blood gases, pH and respiratory mechanics data of healthy control groups

		0	1 h	2 h	3 h	4 h	5 h	6 h
PaO_2	C1	537±17	503±27	500±38	525±18	520±24	548±25	527±25
	C2	531±22	512±18	505±18	527±6	507±25	524±18	511±18
$PaCO_2$	C1	34±3	41±3*	42±3*	43±3*	42±2*	40±3*	40±3*
	C2	36±5	39±4	40±3	43±4	38±3	41±3	40±5
pH	C1	7.48±0.02	7.39±0.05*	7.37±0.03*	7.35±0.02*	7.35±0.01*	7.37±0.02*	7.35±0.01*
	C2	7.45±0.05	7.41±0.05	7.38±0.03*	7.37±0.02*	7.36±0.02*	7.36±0.01*	7.36±0.03*
$Peak_{awp}$	C1	15.6±1.3	17.7±0.9*	18.8±1.2*	18.1±1.2*	17.3±1.0*	16.3±2.0	16.4±1.1
	C2	16.6±1.1	16.8±0.7	16.3±1.0	16.0±0.6	15.6±0.6	15.6±0.8	15.6±0.8
$Pause_{awp}$	C1	13.5±0.5	15.0±1.0*	16.5±1.6*	16.4±1.7*	15.6±1.6*	14.6±2.8	14.8±1.7
	C2	14.8±1.5	15.6±0.9	15.4±1.0	15.0±0.6	14.3±0.4	14.5±0.9	14.4±0.8
$Mean_{awp}$	C1	5.6±0.2	6.4±0.1*	6.4±0.1*	6.3±0.2*	6.0±0.2*	5.6±0.5	5.6±0.4
	C2	5.8±0.4	5.8±0.4#	5.6±0.5#	5.5±0.3#	5.5±0.3	5.4±0.4	5.4±0.4

Data are mean SD.
PaO_2 = Arterial oxygen tension; $PaCO_2$ = Arterial carbon dioxide tension; $peak_{awp}$ = peak airway pressure (cm H_2O); $pause_{awp}$ = end-inspiratory airway pressure (cm H_2O); $mean_{awp}$ = mean airway pressure (cm H_2O); C1 = perfluorocarbon-treated; C2 = only ventilated.
* Statistically different ($p<0.05$) from baseline value (0 point).
# Statistically different ($p<0.005$) from perfluorocarbon-treated group (C1).

DISCUSSION

This study demonstrated at least three points of practical interest regarding mechanical ventilation in combination with intratracheal perfluorocarbon administration. First, it suggested that pulmonary gas exchange can be improved by increasing the administered perflubron dose in animals with acute respiratory failure secondary to surfactant depletion. Second, airway pressures can be decreased and respiratory system compliance can be improved to almost the same extent at all perflubron doses. Finally, and most importantly, it suggests that these beneficial effects can be maintained for several hours, again in a dose-dependent fashion. In other words, this study demonstrates a time-dependent characteristic of the four tested perflubron doses in their ability to maintain the improvements in lung functions in acute respiratory failure induced-animals. A dose-dependent relationship for pulmonary gas exchange, but not for respiratory mechanics, indicates different mechanisms of action of intratracheal perflubron administration in diseased lungs.

Alveolar collapse, resulting from increased alveolar surface tension, is one of the main pathophysiologic features of RDS that lead to intrapulmonary shunt. It normally can be prevented by high end-expiratory pressures. Perfluorocarbon liquids have low surface tensions, which make them potentially useful in liquid ventilation in RDS. We speculate that, following intratracheal administration of even a low dose of perflubron, alveoli and airways in the whole lung are covered with a perflubron film; therefore, the increased surface tension at the air-liquid interface is reduced to that of the perflubron liquid. This mechanism allows for relatively low pressure lung inflation. Our experimental results

support this hypothesis, in that the airway pressures were reduced independently of the dose administered.

In view of the dose-related improvement of oxygenation, we suggest that, despite the reduction of alveolar surface tension by perflubron in diseased lungs, this low but constant surface tension still does not prevent alveoli from end-expiratory collapse at a PEEP of 6 cm H_2O. The improvement of gas exchange at increasing doses, and therefore the recruitment of previously collapsed units (presumably the nonfilled alveoli in the upper lung regions), can be attributed only to the amount of the administered

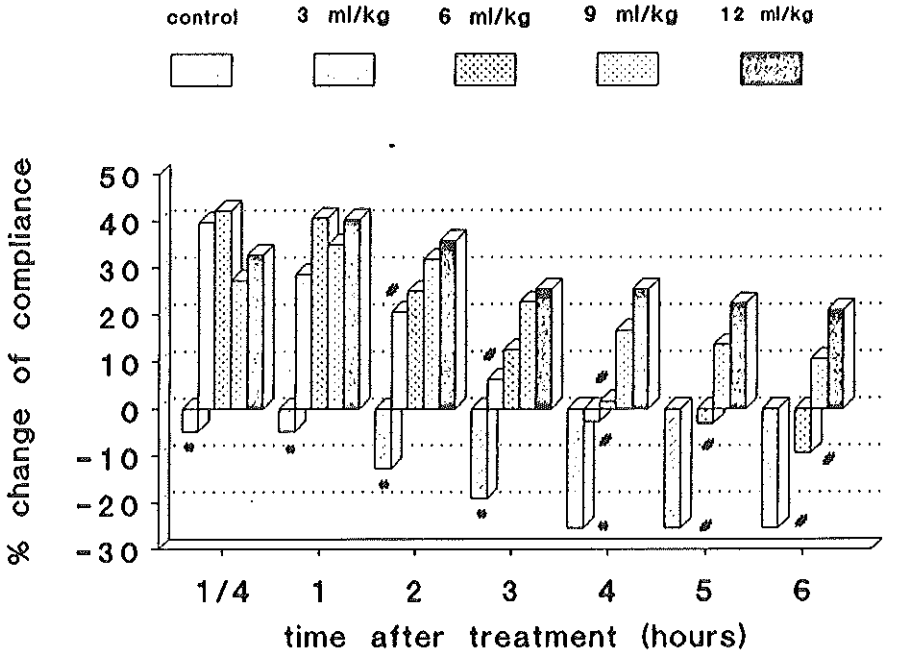

Figure 4. Percentage changes from the pretreatment level in lung compliance over time in the treatment groups. Following repeated lung lavage, there was marked decrease in lung compliance in all groups in the range of 33-42% (not shown in the figure). * = significantly different ($P < 0.0001$) from other groups; # = significantly different ($P < 0.0001$) from 9 and 12 $ml \cdot kg^{-1}$ groups.

perfluorocarbon liquid, (i.e., the administration of high volumes of perfluorocarbon results in the filling of more affected alveoli). This "splinting" of alveoli prevents end-expiratory collapse so that gas exchange can be facilitated during the whole respiratory cycle. Perflubron's ability to dissolve large amounts of oxygen and carbon dioxide results in continuous alveolar gas exchange and decreased intrapulmonary shunt.

After filling the lungs with high doses of perfluorocarbon (>12 $ml \cdot kg^{-1}$) and maintaining a gas tidal volume above this volume, one would expect high airway pressures to expand the lungs. To clarify this hypothesis, we performed an additional experiment for dynamic pressure-volume measurements in lung-lavaged animals. Table 4 indicates that, in RDS-induced animals at a PEEP level of 6 cm H_2O, gas tidal volume was maintained at considerably lower airway pressures, even after filling the lungs with a perflubron volume of 20 $ml \cdot kg^{-1}$, than only gas ventilation state (0 point). At increasing doses of perflubron (up to 40 $ml \cdot kg^{-1}$), gas ventilation resulted in high peak airway pressures. Even though this result may reflect the compression of gas tidal volume in a lung largely full of perfluorocarbon liquid, the data suggest that the alveoli were nearly at full expansion, at which point the tissue elasticity became the predominant component of alveolar retractive forces, thereby leading to significant airway pressure changes. This mechanical behavior of the lavaged lungs with increasing perflubron volumes resembled the pressure-volume characteristics of saline-filled lung[14].

Perfluorocarbon liquids are metabolically inert and are eliminated via the expired air. Although systemic absorption and distribution of perfluorocarbon to the blood and other tissues in small amounts have been demonstrated after liquid ventilation, measurements of perfluorocarbon in expired gases have documented the rapid

elimination of perfluorocarbon through the lungs.[15,16] The relationship between the dose of perfluorocarbon administered and the time at which impairment of lung function was seen strongly supports the suggestion that evaporation of perfluorocarbon over time would cause the affected alveoli to collapse and, therefore, limit efficacy of pulmonary gas exchange. This will occur sooner with small doses of perfluorocarbon, as observed in this study.

TABLE 4. Dynamic volume-pressure measurements

PFC volume ($ml \cdot kg^{-1}$)	Pressure (cm H_2O)
0 (after lavage)	25.0 ± 1.5
5	18.9 ± 0.4
10	18.9 ± 1.4
15	19.4 ± 1.8
20	21.6 ± 3.1
25	24.1 ± 3.9
30	28.2 ± 5.4
35	32.2 ± 6.0
40	37.2 ± 7.9

Data are mean ± SD from three lung-lavaged and volume-controlled ventilated rabbits (with the same experimental protocol). The pressure represents the end-inspiratory airway pressure recorded at zero flow after five breaths of gas tidal ventilation (12 ml/kg, 100% oxygen) after administration of each perflubron dose in 5 $ml \cdot kg^{-1}$ increments.

Another possibility could be damage of the alveolar structure by the perfluorocarbon liquid itself. In liquid ventilation studies in healthy animals, a reversible change in pulmonary function has been observed and speculated to be caused by the

residual perfluorocarbon in the lungs rather than the pathophysiologic changes.[17-19] Histologic studies in preterm animals have revealed that after ventilation with perfluorocarbon liquids, the alveolar structure remained intact and no damage to the lung architecture was demonstrated compared to conventional gas ventilation.[2,20,21] After filling the lungs with perfluorocarbon at a volume of functional residual capacity, Fuhrman et al. recently demonstrated that adequate pulmonary gas exchange can be provided with conventional mechanical ventilation in healthy animals.[22] Our results from healthy animals are in agreement with Fuhrman's report that healthy animals can tolerate intratracheal perfluorocarbon administration during mechanical ventilation without showing any clinically evident harmful effects on lung functions.

We can not exclude the possibility that the proteinaceous exudate elicited by the saline lavage could mix with the perfluorocarbon and, in the groups receiving low-dose perfluorocarbon, reduce its surface tension effect by dilution; however, combined with the aforementioned reports, the present experimental data from healthy controls, which revealed no harmful effects caused by perflubron itself or the time factor involved, suggest that elimination of perfluorocarbon by evaporation through the lungs remains the major determinant of efficacy of this ventilatory support technique. An important observation was that significant impairment of gas exchange occurred earlier than did the increase in airway pressures. This observation also indirectly supports our hypothesis that perfluorocarbon acts by different mechanisms for oxygenation and lung mechanics properties in the diseased lung. In the groups treated with 3 $ml \cdot kg^{-1}$ and 6 $ml \cdot kg^{-1}$ of perfluorocarbon, animals developed pneumothorax at the time points where the peak airway pressures were greater than the pretreatment levels. However, end-inspiratory

airway pressures never exceeded the pretreatment inflation pressures in the groups treated with 9 ml•kg^{-1} and 12 ml•kg^{-1} of perfluorocarbon (6% and 12% less than the pretreatment levels at 6 h, respectively) and no pneumothorax occurred in these groups. Therefore, we may conclude that cyclic reopening and collapse of the alveoli and airways with high pressure amplitudes could lead to high shear forces along the epithelial surfaces and, thus, result in barotrauma.[23] In addition to well sustained lung mechanics (reduced airway pressures, increased respiratory system compliance), maintenance of adequate pulmonary gas exchange for several hours suggests that this technique offers an effective means of ventilatory support with high perfluorocarbon doses and minimizes the risk of barotrauma. Considering the experimental results, it can be suggested that the progress of functional lung impairment due to evaporation of perfluorocarbon can be prevented by replacement of perfluorocarbon liquid at the time that decrease in efficacy is observed.

It is noteworthy that the vapor pressure of a particular perfluorocarbon could be important for both producing efficient evaporation from the lungs at the termination of this supportive technique and avoiding frequent replacement during long-term applications used to decrease the cost. Moreover, the vapor pressure of perfluorocarbon appears to be an important determinant for the theoretical alveolar oxygen level. The perfluorocarbon tested in this experiment, perflubron, has a low vapor pressure of 10.5 mm Hg at 37°C and 3.6 mm Hg at 20°C. Therefore, the presence of saturated perflubron vapor would only decrease the maximal alveolar oxygen pressure by 10.5 mmHg, much less than would be expected when other more volatile perfluorocarbons were employed. The low vapor pressure makes perflubron the preferred perfluorocarbon for this type of

application, with respect to evaporative losses and maintenance of alveolar oxygen tensions.

Before clinical application is undertaken, perfluorocarbon liquids must be shown to be safe regarding side effects, tissue retention, and toxicity. A considerable number of animal toxicology studies have been performed on perflubron, which has been used also in humans as an oral magnetic resonance contrast agent for the gastrointestinal tract.[24,25] Moreover, perflubron is presently in clinical trials in the United States as an intravenous contrast agent for blood pool imaging with computed tomography, and the limited data have shown no significant side effects.[26]

The development of lactic acidosis is thought to reflect an imbalance between the metabolic requirements and oxygen supply to the tissue. Despite the small, insignificant changes in metabolic rate of the animals as evaluated by carbon dioxide production, lactic acidosis (unrelated to the perflubron dose) developed in the treatment groups. Considering the limited available data, the occurrence of lactic acidosis is likely to be a reflection of cardiovascular dysfunction, because uncomplicated hypoxemia does not cause lactic acidosis,[27] and intravascular fluid volume plays an important role in maintaining adequate oxygen supply during positive-pressure ventilation.[28] Further studies are required to evaluate the possible mechanisms of cardiocirculatory adjustments and increased lactate levels during this type of perfluorocarbon application.

In conclusion, intratracheal perflubron administration combined with conventional mechanical ventilation offers an easier and more clinically acceptable approach to perfluorocarbon use compared to total liquid ventilation. It also provides a degree of respiratory support that is unlikely to be achieved with conventional ventilation alone at

the same ventilatory settings. Intratracheal perflubron instillation combined with mechanical gas ventilation provides adequate oxygenation, improves ventilation, and allows for low inflation pressures without compromising hemodynamics in acute respiratory failure. These results indicate that even very low doses of perflubron permit significant improvement in lung function, and higher doses (reaching to functional residual capacity volume) are required for adequate ventilatory support for periods up to 6 h in severe RDS in animals. Exogenous surfactant treatment has been shown to be a promising adjunct in various experimental models and in a limited number of clinical cases of RDS.[29] The present study suggest that intratracheal perfluorocarbon treatment also may play a role in achieving the goals of ventilatory support in humans with RDS (i.e., pulmonary support avoiding high inflation pressures) and, thus, further lung damage, until sufficient recovery from the primary disease is achieved.

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CHAPTER 5

EFFECTS OF PARTIAL LIQUID VENTILATION ON GAS EXCHANGE AND LUNG MECHANICS IN HEALTHY ANIMALS

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EFFECTS OF PARTIAL LIQUID VENTILATION ON GAS EXCHANGE AND LUNG MECHANICS IN HEALTHY ANIMALS

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INTRODUCTION

As a means of ventilatory support, partial liquid ventilation has been investigated by various investigators in animals with acute respiratory failure (1-4). Studies have demonstrated this new technique to improve lung mechanics and respiratory parameters in diseased lungs, with promising results for future clinical application.

Looking at the effects of partial liquid ventilation on healthy animal lungs, there are few studies showing that lung mechanics and gas exchange parameters are well preserved during short-term partial liquid ventilation application (5,6). However, this issue has been more extensively evaluated during total liquid ventilation studies in healthy animals, and a temporary impairment in arterial oxygenation has been commonly reported on returning to gas breathing (7-12). In addition, some reversible changes in lung mechanics parameters (such as increased airway resistance, decreased respiratory system compliance) have been reported following total liquid ventilation (9-11,13). All these changes in reversible character are speculated to be due to residual PFC

remaining in the lung. Despite the extensive body of research on total liquid ventilation, this issue needs to be further investigated with the new technique of partial liquid ventilation, since these two approaches differ technically.

Therefore, we aimed to study the effects of partial liquid ventilation on gas exchange and respiratory mechanics parameters in healthy animals. These parameters were investigated during a partial liquid ventilation trial of 3 hours, and comparisons with the control values were made by the seventh day after partial liquid ventilation to determine the long-term effects on the same parameters.

METHODS

The study was approved by the Animal Committee of Erasmus University Rotterdam.

Animal Preparation:

Six adult New Zealand rabbits with a mean body weight of 2.6 ± 0.2 kg were prepared by avoiding food 12 h before the experiments. Animals were sedated with an IM injection (0.3 ml/kg) of Hypnorm (10 mg fluniason and 0.2 mg fentanyl per ml; Janssen, The Netherlands) 15 min before the induction of anesthesia. Each animal was anesthetized with halothane (1%) and was orotracheally intubated with a cuffed-tube of 3.0-3.5 mm ID. The animals were positioned supine, and volume-controlled ventilation with a Servo Ventilator 900C (Siemens-Elema, Sweden) was initiated using 100% oxygen and zero end-expiratory pressure with a tidal volume (Vt) of 12 ml/kg, respiratory frequency (f) of 30/min and inspiratory/expiratory time ratio (I/E) of 1:2. An auricular

artery was catheterized for arterial blood sampling and an auricular vein was catheterized for fluid administration. Anesthesia was maintained with additional doses of Hypnorm, as required. No muscle relaxant agent was given. Via the auricular vein, 5% dextrose/0.45% NaCl solution was continuously administered at a rate of 7.5 ml/kg/h as a maintenance fluid.

As the baseline measurement, blood gas measurements and respiratory mechanics recordings were made at positive end-expiratory pressure (PEEP) levels of 0, 2 and 6 cm H_2O after ventilation for 15 min at each PEEP level to get a steady-state. Following baseline measurements, a total volume of 12 ml/kg PFC was administered intratracheally in two equal subsequent volumes as bolus (at 3-min intervals). At instillation, animals were disconnected from the ventilator and reconnected immediately following PFC instillation, and voMwme-controlled ventilation at the same settings (FiO_2=1, PEEP=6 cm H_2O, Vt=12 ml/kg, f=30/min and I/E=1:2) was continued for 3 h. During this period, blood gases and pH were determined (ABL-330, Radiometer, Copenhagen, Denmark) and respiratory mechanics (airway pressures, respiratory system resistances) were measured (Lung Mechanics Calculator 940, Siemens-Elema, Sweden) at 15 min and at 30 min intervals thereafter.

At the end of the 3-h observation period, animals were allowed to breathe spontaneously and naloxonhydrochloride IM (0.08 mg/kg) was administered to all animals. When PaO_2 was more than 70 mm Hg at room air, animals were extubated, the catheters were removed and animals were housed in wire-basket cages for 7 days. Animals were fed standard chow, ad libitum. On day 7, the animals were anesthetized, intubated and mechanically ventilated according to the same above-mentioned protocol;

blood gas measurements and respiratory mechanics recordings were performed at PEEP levels of 0, 2 and 6 cm H_2O. Animals were then sacrificed with an overdose of pentobarbital.

Treatment:

All studies were performed using Liquivent™ (perflubron [perfluoro-octyl bromide]; Alliance Pharmaceutical Corp, San Diego, Ca, USA). Perflubron is a PFC with a specific gravity of 1.918 g/cm^3 at 25°C, surface tension of 18.1 dynes/cm, vapor pressure of 3.6 mm Hg at 20°C and 10.5 mm Hg at 37°C, O_2 solubility of 53 ml/100 ml and CO_2 solubility of 210 ml/100 ml at 37°C and 1 atmosphere pressure.

Statistical Analysis:

All data are expressed as mean ± SD. The data for non-repetitive measurements were analyzed by Student's t-test. The repeated measurements were compared by multifactor analysis of variance (ANOVA) using the multiple range test. P values less than 0.05 were considered as statistically different.

RESULTS

All animals tolerated the 3-h mechanical ventilation after intratracheal administration of PFC and were easily reconverted to spontaneous breathing. The extubation time (from completion of the observation period) ranged between 5 and 15 min in all animals. Before extubation, there was no visible recovery liquid (PFC) coming out of the tracheal tube at disconnection from the ventilator. No adverse effects attributable to partial liquid ventilation were detected in the spontaneous breathing pattern of the animals (e.g. no

wheezing or cough) and all animals survived the 7-day study period.

As shown in Table 1, there were no significant differences in arterial blood chemistry at increasing PEEP levels. After instillation of PFC, the pulmonary gas exchange and acid-base status of the animals did not change and remained stable for 3 h compared to the baseline values.

The airway pressures needed to inflate the lungs remained unchanged after PFC administration (Table 1). The only significant change in lung mechanics occurred at airway resistances such that respiratory resistance at inspiration was significantly higher, while respiratory resistance at expiration was lower during partial liquid ventilation compared to pretreatment level.

Table 1. Arterial blood gases (mm Hg), pH and respiratory mechanics data before, during and 7 days after ventilation with intratracheal perfluorocarbon at different PEEP levels (mean±SD).

	Before			During				After		
	PEEP 0	PEEP 2	PEEP 6	15 min	1 h	2 h	3 h	PEEP 0	PEEP 2	PEEP 6
PaO_2	569±57	560±49	577±30	529±27	555±36	572±50	571±46	549±28	557±24	575±17
$PaCO_2$	32±5	30±5	29±3	30±4	30±3	31±5	31±5	32±4	32±3	30±4
pH	7.45±.08	7.49±.07	7.50±.06	7.51±.07	7.54±.05	7.52±.06	7.49±.07	7.53±.08	7.52±.09	7.51±.07
$Peak_{awp}$	11.8±1.5	14.0±1.5	16.1±1.0	16.1±0.9	16.5±1.1	16.3±1.1	16.7±1.8	12.1±1.1	13.7±1.0	17.1±1.3
$Pause_{awp}$	10.3±1.6	12.3±1.7	14.6±1.0	14.4±0.8	14.7±1.0	14.5±1.0	14.9±1.7	11.0±0.8	12.9±1.0	16.3±1.3
$Mean_{awp}$	3.5±0.4	4.9±0.3	8.5±0.4	8.6±0.4	8.7±0.4	8.6±0.4	8.7±0.5	3.5±0.3	5.0±0.3	8.6±0.3
R_{insp}	26±7	23±8	19±4	32±5*	32±6*	33±6*	33±10*	26±3	22±4	17±4
R_{exp}	82±10	98±5	112±2	81±10*	81±11*	81±14*	85±12*	84±7	99±12	111±4

$Peak_{awp}$=peak airway pressure (cm H_2O); $Pause_{awp}$=end-inspiratory airway pressure (cm H_2O); $Mean_{awp}$=mean airway pressure (cm H_2O); R_{insp}=respiratory resistance at inspiration (cm H_2O/L/sec); R_{exp}=respiratory resistance at expiration (cm H_2O/L/sec).
*=statistically different ($p<0.005$) by ANOVA test compared with pretreatment value at a PEEP of 6 cm H_2O.

At day 7 after the trial, the mean data for pulmonary gas exchange and respiratory system mechanics at PEEP levels of 0, 2 and 6 cm H_2O were comparable with the control pretreatment data (Table 1).

DISCUSSION

This study demonstrated that partial liquid ventilation can be successfully applied to healthy animals with uncomplicated reconversion to gas breathing and, moreover,respiratory parameters (arterial blood gases and lung mechanics) remain unchanged at the long-term period (by the 7th day) following 3 h of partial liquid ventilation.

Partial liquid ventilation is a new ventilatory support technique which combines gas tidal ventilation and intratracheal PFC administration. The first experimental report on this technique in healthy animals was recently documented by Fuhrman et al. (5). In their study, they demonstrated that, after filling the lungs to functional residual capacity (FRC) volume with PFC, oxygenation and ventilation can be as effective with partial liquid ventilation as with conventional mechanical ventilation during the 1 h trial period. Our present experimental results demonstrating adequate oxygenation during partial liquid ventilation are consistent with that of Fuhrman et al. Furthermore, in contrast to the adequate but decreased PaO_2 in Fuhrman's study, better arterial oxygenation was achieved and maintained for 3 h with a PFC volume less than the FRC volume (considering the FRC volume of healthy rabbits as 18 ml/kg) in the present study. In other words, arterial pO_2 was above 500 mm Hg throughout the study, reflecting a

complete participation of alveolar units during gas exchange during both inspiration and expiration. This level of oxygenation suggested that even at a PEEP level of 6 cm H_2O, the PFC-filled lungs were operating at safe lung volumes with the superimposed gas tidal volumes where lung overdistension was not expected to occur. Besides, arterial pCO_2 was consistently stable at control levels for 3 h as well. Therefore, the preservation of arterial oxygenation and ventilation at control levels throughout the 3-h study period reflected a perfect matching of alveolar ventilation and perfusion during partial liquid ventilation.

In this study, the presence of PFC did not produce significant changes in mechanical properties of the lung during partial liquid ventilation. The airway pressures at both inspiration and end-inspiration were identical to the control values during partial liquid ventilation. In other words, even after filling the lungs with a PFC volume of 12 ml/kg, the airway pressures required to inflate the lungs with a gas tidal volume of 12 ml/kg were not significantly different when compared to only gas ventilation state (16.7 ± 1.8 vs 16.1 ± 1.0 cm H_2O, respectively). Therefore, it is reasonable to speculate that due to the low surface tension characteristic (18.1 dynes/cm), PFC is easily distributed in the lung and alveoli could be easily distended with gas tidal volumes despite the increased inspiratory airway resistance. This mechanical behavior of healthy lungs are in agreement with that of Fuhrman's report.

Following 3 h of partial liquid ventilation, all animals could be successfully weaned off the mechanical ventilation and spontaneous breathing could be resumed. The weaning from controlled ventilation to unassisted breathing took no more than 15 min in each animal and adequate gas exchange could be achieved at room air.

Several investigators have reported successful reconversion to gas breathing following total liquid ventilation (7-12). However, a temporary impairment in arterial oxygenation and some reversible changes in lung mechanics parameters (increased airway resistance, decreased respiratory system compliance with increased airway pressures) have been reported on returning to gas breathing following total liquid ventilation (7-13). All these changes in reversible character are speculated to be due to residual PFC liquid remaining in the lungs. Recently, Salman et al. studied gas exchange and lung mechanics during evaporation of PFC following PLV in healthy animals (6). In contrast to the present study, they demonstrated that an adequate level of oxygenation could be maintained at the cost of an increased level of PEEP following partial liquid ventilation. The important point for consideration in their study is that the lung histology did not correlate with the laboratory data. Despite the normal preservation of lung histology at 24 h following PFC administration, airway pressures needed to be increased to keep oxygenation stable, unlike the present study.

In our study, evaluation of the mechanics of the respiratory system for long-term effects after partial liquid ventilation was performed by PEEP titration. By keeping the tidal ventilation constant, measurement of airway pressures at increasing PEEP levels allows determination of the pressure-volume characteristics of the respiratory system. Since the inflation pressure at zero flow (end-inspiratory pause period) reflects mainly an estimate of alveolar pressure, the unchanged inflation pressures at corresponding identical PEEP levels one week after partial liquid ventilation trial, in comparison to pretreatment control levels, reflected the preservation of the elastic forces (surface tension and tissue properties) along the alveolar units in the lung. Within the range of

0-6 cm H_2O of PEEP, the pressure-volume relationship of PFC-treated animals in this study was not changed by the seventh day following partial liquid ventilation. That is, the static compliance was calculated as 3.15±0.31, 3.16±0.52 and 3.26±0.37 ml/cm H_2O before partial liquid ventilation, and 3.03±0.19, 3.06±0.30 and 3.25±0.55 ml/cm H_2O after partial liquid ventilation at corresponding PEEP levels of 0, 2 and 6 cm H_2O, respectively.

The changes in pulmonary gas exchange and lung mechanics in healthy animals following total liquid ventilation were observed to return to preliquid breathing levels within several days. Thus, the question whether the animals in the present study experienced the same changes during the one-week observation period remains unanswered. It is likely that the reported alterations and the time course of these changes in lung mechanics and gas exchange upon return to gas ventilation after liquid ventilation may be influenced by the different physico-chemical properties of different PFC liquids, such as vapor pressure and surface tension. Nevertheless, the present experimental data by the seventh day revealed that there were no clinically evidenced changes on lung functions in terms of gas exchange and respiratory mechanics following partial liquid ventilation. On the other hand, in various histologic studies in healthy animals, alveolar structures appeared to be histologically normal within a few days (8,14,15) and remained normal even several months after liquid ventilation (8,10,14-16). Additionally, in a morphologic study, the ultrastructure of the lung remained unchanged after liquid ventilation (17).

The elimination of intratracheal PFC is mainly by evaporation via the lungs. Although PFC is absorbed and distributed in the tissues in small quantities and persists for several years after intratracheal instillation (16), it has been shown that expiratory PFC

level is at control levels at 8 h following liquid ventilation in human neonates (18). Despite the presence of residual PFC in various tissues, studies in both human neonates and animals demonstrated that residual PFC was not associated with any evidence of adverse reaction in any of the tissues (16,19). In this study, we have not made expiratory PFC measurements. However, the animals were free of visible recovery liquid and clinically stable at extubation following the PFC trial. The mechanisms for tissue uptake, distribution and elimination of PFC liquid are not yet clearly defined and further investigation is warranted.

In conclusion, the present experimental data demonstrates that healthy animals tolerate partial liquid ventilation with unchanged pulmonary gas exchange and respiratory system mechanics both during and after this type of ventilatory support. However, the effects of partial liquid ventilation application on lung function and structure in healthy states remain an important point for consideration, particularly with respect to the application of different PFC liquids at different volumes and the duration of partial liquid ventilation. Considering the potential use of this technique in clinical conditions, these issues need to be further evaluated with more sensitive and clinically reliable measures.

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CHAPTER 6

EVALUATION OF LUNG FUNCTION AFTER INTRATRACHEAL PERFLUOROCARBON ADMINISTRATION IN HEALTHY ANIMALS: PULMONARY CLEARANCE OF ^{99m}Tc-DTPA

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EVALUATION OF LUNG FUNCTION AFTER INTRATRACHEAL PERFLUOROCARBON ADMINISTRATION IN HEALTHY ANIMALS: PULMONARY CLEARANCE OF ^{99m}Tc-DTPA

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ABSTRACT

Objectives: To investigate the effects of partial liquid ventilation, i.e. mechanical ventilation in combination with intratracheal administration of perfluorocarbon, on lung function with particular interest on the integrity of the alveolo-capillary membrane in healthy adult animals.

Design: Prospective, randomized, controlled study.

Setting: Labarotory at the Department of Experimental Anesthesiology, Erasmus University Rotterdam.

Subjects: Ten adult male New Zealand rabbits.

Interventions: Five rabbits were intratracheally treated with 12 mL/kg of perfluorocarbon while conventional mechanical ventilation (volume-controlled, tidal volume = 12 mL/kg, respiratory frequency = 30/min, inspiratory to expiratory time ratio = 1:2,

PEEP = 2 cm H_2O and 100% oxygen) was applied for 3 h. To assess the permeabilty of the alveolo-capillary membrane, pulmonary clearance of inhaled technetium-99m-labelled diethylene triamine pentaacetic acid (^{99m}Tc-DTPA) measurements were performed at 3 h and compared with data from the control group (n = 5) treated with mechanical ventilation only using the same ventilatory parameters.

Measurements and Main Results: Pulmonary gas exchange and lung mechanic parameters were measured in both groups at 30 min intervals. Although at adequate levels, mean values for PaO_2 in the perfluorocarbon group were less than those of the control group during the 3-h study period (370 ± 44 torr vs 503 ± 44 torr at 3 h)(49.3 ± 5.9 kPa vs 67.1 ± 5.9 kPa). Peak and mean airway pressures were higher in the perfluorocarbon group (ranging from 1.9 - 3.4 cm H_2O and 0.7 - 1.3 cm H_2O, respectively) compared to the control group, while end-inspiratory airway pressure was similar in both groups. The half-life of ^{99m}Tc-DTPA was 83.7 ± 24.5 min in the control group, which was significantly longer ($p < 0.01$) than in the perfluorocarbon group (49.8 ± 6.1 min).

Conclusions: These findings suggest that partial liquid ventilation with perfluorocarbons lowers pulmonary gas exchange in healthy animals, and the increased pulmonary clearance of ^{99m}Tc-DTPA after 3 h of this type of ventilatory support may reflect minimal reversible changes in the lung surfactant system.

INTRODUCTION

Studies with perfuorocarbon total liquid ventilation have shown this technique to

be an effective means of respiratory support to provide effective pulmonary gas exchange and to improve lung function in animals with acute respiratory failure (1-5). As an alternative perfluorocarbon application method to those requiring a liquid ventilation system in these referenced studies, we recently demonstrated that intratracheal perfluorocarbon administration in combination with conventional mechanical ventilation (partial liquid ventilation) improves pulmonary gas exchange in a dose-related manner in an animal model of acute respiratory failure (6). More recent studies using the saMw approach indicate that effective respiratory support can be maintained for several hours at high perfluorocarbon doses (7) and, furthermore, lung structure is well maintained in animals with acute respiratory failure (8).

Looking at the effects on healthy lungs, pulmonary gas exchange, lung mechanics and cardiovascular dynamics were investigated in various studies in healthy animals using total liquid ventilation. Although at satisfactory levels, arterial oxygenation was consistently lower in healthy animals during total liquid ventilation. At present, partial liquid ventilation in healthy lungs is limited to only a few animal experiments. Using this new approach of ventilatory support, Fuhrman et al. demonstrated that after filling the lungs with perfluorocarbon to a volume of functional residual capacity, adequate pulmonary gas exchange can be provided in healthy animals (9).

Since this new technique differs from total liquid ventilation, and employs conditions applied to both liquid and gas physics, this technique of partial liquid ventilation needs to be further investigated with more sensitive and detailed methods, especially with respect to its safety for normal lung function and also compared to other available ventilatory support techniques.

Because integrity of the alveolo-capillary barrier is an important factor for normal function of the respiratory system, there is great interest in measures investigating any changes to this barrier function, such as measurement of pulmonary clearance of radio-labelled hydrophilic solutes like technetium-99m-labelled diethylene triamine pentaacetic acid (^{99m}Tc-DTPA). Measurement of pulmonary clearance of ^{99m}Tc-DTPA is proven to be a very sensitive, fast and non-invasive technique to detect the integrity of the alveolo-capillary barrier (10,11). Moreover, it has been demonstrated that the clearance rate of ^{99m}Tc-DTPA mainly depends on the functional integrity of the surfactant system (12,13). Thus, in order to evaluate the effects of partial liquid ventilation on lung function (gas exchange and lung mechanics), and specifically on the integrity of the alveolo-capillary barrier, we tested the clearance of ^{99m}Tc-DTPA from the lungs in healthy animals after 3 h of partial liquid ventilation and compared the data with conventional gas ventilation.

METHODS

This study was approved by the Animal Committee of Erasmus University Rotterdam.

Animal Preparation:

Ten adult New Zealand rabbits with a mean body weight of 2.2 ± 0.2 kg were anesthetized with an IV injection of pentobarbital sodium (50 mg/kg) via an auricular vein. The animals were positioned supine, tracheotomized, and volume-controlled ventilation with a Servo Ventilator 900C (Siemens-Elema, Sweden) was initiated with tidal volume of 12 mL/kg, respiratory frequency of 30/min, inspiratory to expiratory time ratio of 1:2,

positive end-expiratory pressure (PEEP) of 2 cm H_2O and 100% oxygen. Anesthesia was maintained with additional doses of pentobarbital, as required; pancuronium bromide was administered by continuous infusion (0.1 mg/kg/h) for muscle paralysis. A maintenance fluid of 5% dextrose 0.45% NaCl was administered (7.5 mL/kg/h). A carotid artery was cannulated for blood pressure monitoring and blood sampling.

Following animal preparation and baseline measurements, animals were randomized into two groups of 5 animals each. In one group, a volume of 12 mL/kg perfluorocarbon was administered intratracheally in two subsequent volumes as bolus (at 3 min intervals). At instillation, animals were disconnected from the ventilator and reconnected immediatelly following perfluorocarbon administration and volume-controlled ventilation was continued. The second group received no treatment but was mechanically ventilated only. The study protocol was limited to 3 h, because the animalsMwere observed to be free of visible perfluorocarbon at the endotracheal tube during disonnection from the ventilator at 3 h (unpublished observations). Both groups were ventilated for 3 h, keeping the ventilator settings same as above: volume-cotrolled, FiO_2 = 1, tidal volume = 12 mL/kg, respiratory frequency = 30/min, inspiratory to expiratory time ratio = 1:2 and PEEP = 2 cm H_2O.

During the study period, blood gases and pH were measured (ABL-330, Radiometer, Copenhagen, Denmark), and respiratory mechanics (airway pressures, airway resistances) were recorded by a Lung Mechanics Calculator 940 (Siemens-Elema, Sweden) (14) at 15 min and at 30 min intervals thereafter. At the end of 3-h observation period, clearance measurements of ^{99m}Tc-DTPA were performed.

Treatment:

All studies were performed using Liquivent™ (perflubron [perfluorooctyl bromide]; Alliance Pharmaceutical Corp, San Diego, USA). Perflubron is a perfluorocarbon with a specific gravity of 1.918 g/cm^3 at 25°C, surface tension of 18.1 dynes/cm, vapor pressure of 3.6 torr (0.5 kPa) at 20°C and 10.5 torr (1.4 kPa) at 37°C, O_2 solubility of 53 mL/100 mL and CO_2 solubility of 210 mL/100 mL at 37°C and 1 atmosphere pressure.

Clearance Measurements:

The animals were placed under a gamma camera and were kept under the same anesthesia protocol as above. Radioactivity was measured in the anterior view.

A solution of ^{99m}Tc-DTPA, prepared from a commercial kit (Technescan DTPA, Mallinckrodt Medical, Petten, The Netherlands), was nebulized into the inspiratory line of the ventilation circuit as described by Dahlbäck (15), using an air jet nebulizer (Ultravent, Mallinckrodt Medical, The Netherlands). This type of nebulizer is designed to produce aerosols with droplets in the range of 0.6 - 2μm, favoring alveolar deposition (16). The supply of pressurized air to the nebulizer was controlled by a pneumatic valve which was connected to the ventilator with an eloctronic circuit. The nebulizer operated only during expiration, filling the inspiratory line with aerosol in order to administer the particles with the ensuing insufflation. Before running the nebulizer, in each animal the system was checked for air leaks. During aerosol administration, all animals in both groups were pressure-controlled ventilated with a peak inspiratory pressure equal to the final recording of end-inspiratory airway pressure during volume-controlled ventilation, and respiratory frequency of 30/min, inspiratory to expiratory time ratio of 1:1 and zero end-expiratory pressure were used. These ventilator settings are demonstrated to create an

intrinsic PEEP of 2 cm H_2O (identical to the experimental set-PEEP level) and a high radioactivity could be reached over the lungs within 1-2 min (17). When a count rate of approximately 300 - 400 counts per second over the lungs was obtained, aerosol administration was discontinued, the experimental ventilation settings (volume-controlled) were resumed and clearance measurements were started immediately. Gamma camera images were obtained in successive 1-min frames for 20 min and stored in a computer (Digital PDP 11/34, Maynard, USA).

Data from the clearance measurements from both lungs of each animal were analyzed and a time-activity curve was generated. A mono-exponential function was fitted to the experimental data and the half-life (T1/2) of the tracer in the lungs was calculated. After completion of the measurements, animals were killed with an overdose of pentobarbital.

Statistical Analysis:

All data are expressed as mean ± SD. The repeated measurements were compared by multifactor analysis of variance (ANOVA), using multiple range test. The data for non-repetitive measurements were analyzed by Student's t-test. P values less than 0.05 were considered as statistically different.

RESULTS

All animals survived the study period. The baseline values for the measured variables were comparable in both groups.

During 3-h mechanical ventilation with intratracheal perfluorocarbon administration,

PaO_2 values decreased (27%) compared to baseline values and the mean data for PaO_2 were significantly lower compared to the control group throughout the study period (Table 1). Arterial pCO_2 and pH data were comparable in both groups, with slightly higher $PaCO_2$ and lower pH values in the perfluorocarbon group (Table 1). The mean data for arterial pressures and heart rate remained unchanged in both groups.

Data for respiratory system mechanics are given in Table 2. Peak airway pressure increased following perfluorocarbon administration and led to a slight increase (range 0.7 - 1.3 cm H_2O) in mean airway pressure compared to the control group. The end-inspiratory pause airway pressure was comparable in both groups during the study period. The inspiratory airway resistance in the perfluorocarbon group was significantly higher compared to the control group, whereas expiratory airway resistance values did

Table 1. Arterial blood gases, pH and hemodynamic data of the perfluorocarbon (P) and control (C) groups (mean ± SD).

		Baseline	15 min	60 min	90 min	120 min	150 min	180 min
PaO_2	P	506±39	369±48*	361±36*	367±28*	369±16*	367±34*	370±44*
	C	513±12	510±29#	515±33#	523±32#	532±42#	500±43#	503±44#
$PaCO_2$	P	38±2	43±3	49±6*	50±6*	50±6*	51±7*	51±7*
	C	40±6	37±6	40±4	42±6	42±5	43±5	43±5
pH	P	7.44±0.04	7.43±0.04	7.38±0.04	7.38±0.04	7.36±0.03*	7.35±0.04*	7.34±0.03*
	C	7.42±0.04	7.47±0.04	7.44±0.04	7.40±0.05	7.39±0.03	7.38±0.02	7.38±0.03
BP	P	88±17	90±18	93±18	90±16	88±20	91±13	99±12
	C	84±15	78±8	84±13	88±13	91±16	90±16	93±17
HR	P	328±22	315±20	312±26	308±18	305±21	300±25	301±18
	C	330±15	323±18	314±26	322±20	315±16	318±16	310±17

BP = mean arterial pressure (mm Hg); HR = heart rate (beats/min); 1 torr = 0.1333 kPa.
* = $p < 0.05$ compared with the baseline value.
# = $p < 0.05$ compared with the perfluorocarbon group.

Table 2. Respiratory system mechanics of the perfluorocarbon (P) and control (C) groups (mean ± SD).

		Baseline	15 min	60 min	90 min	120 min	150 min	180 min
$Peak_{awp}$	P	11.9±1.0	14.9±0.8*	14.5±0.9*	14.5±0.6*	14.3±0.6*	14.6±0.7*	14.5±0.5*
	C	10.8±1.7	11.5±1.8#	11.6±1.5#	11.9±1.5#	12.3±1.2#	12.4±1.3#	12.6±1.3
$Pause_{awp}$	P	11.1±1.1	13.2±0.8*	12.8±0.8*	12.9±0.5*	12.7±0.4*	12.8±0.3*	12.7±0.3*
	C	9.5±2.1	10.4±2.3	10.9±1.8	11.0±1.9	11.5±1.6	11.7±1.4	11.8±1.3
$Mean_{awp}$	P	4.5±0.2	5.8±0.2*	5.5±0.2*	5.6±0.2*	5.5±0.2*	5.5±0.2*	5.5±0.1*
	C	4.3±0.5	4.5±0.4#	4.6±0.5#	4.6±0.4#	4.7±0.4#	4.8±0.4#	4.8±0.4#
R_{insp}	P	24±3	39±6*	34±4*	35±4*	37±5*	38±5*	39±5*
	C	28±8	28±6	26±11	26±11	24±7#	24±5#	24±6#
R_{exp}	P	101±16	102±14	98±15	105±13	105±15	107±13	108±10
	C	97±16	104±11	109±6	110±5	114±1	114±1	112±5

$Peak_{awp}$ = peak airway pressure (cm H_2O); $Pause_{awp}$ = end-inspiratory pause airway pressure (cm H_2O); $Mean_{awp}$ = mean airway pressure (cm H_2O); R_{insp} = respiratory resistance at inspiration (cm H_2O/L/sec); R_{exp} = respiratory resistance at expiration (cm H_2O/L/sec).
* = $p < 0.05$ compared with the baseline value.
# = $p < 0.05$ compared with the perfluorocarbon group.

not differ between the two groups.

Distribution of the nebulized ^{99m}Tc-DTPA aerosol in the lungs was uniform on anolog images in all animals. The clearance curves of ^{99m}Tc-DTPA from the two groups are shown in Figure 1. The faster pulmonary clearance of the tracer is seen in the time-activity curve of the perfluorocarbon group. The mean T1/2 of the tracer in the control group was 83.7 ± 24.5 min (range 54.9 - 125.3 min) which was significantly greater than the mean T1/2 in the perfluorocarbon group, 49.8 ± 6.1 min (range 42.9 - 59.5 min).

DISCUSSION

This study provides the first experimental data on assessment of alveolo-capillary barrier function by means of pulmonary clearance measurements with an aerosol radio-labelled tracer after administering a perfluorocarbon liquid into healthy animal lungs, and demonstrates an increased clearance rate of ^{99m}Tc-DTPA after 3 h of partial liquid ventilation compared to mechanical ventilation only.

The study demonstrates that mechanical ventilation in combination with perfluorocarbon alters pulmonary gas exchange in healthy animals. Our observation that arterial oxygenation is decreased during partial liquid ventilation is consistent with that of the first report with this new ventilatory support technique in healthy animals documented by Fuhrman et al. (9). Although also at adequate oxygen levels, Fuhrman's group demonstrated similar decreases in arterial oxygenation when compared to conventional gas ventilation.

In healthy lungs, the alveolo-capillary barrier (which consists of alveolar surfactant layer, alveolar epithelium, basement membrane and capillary endothelium) protects the transfer of solutes and proteins across the barrier. However, when the integrity of this barrier is altered, permeability is increased, allowing high permeability edema to develop (18). Previous studies indicate that the integrity of the alveolo-capillary membrane is disturbed in patients with adult or infant respiratory distress syndome, and clearance rates of the radiotracers corrolate well with the stage (improvement) of the lung pathology (19,20). The clearance rate of ^{99m}Tc-DTPA has been reported to be changed by various factors, such as in response to altered surface tension in the lungs (21,22),

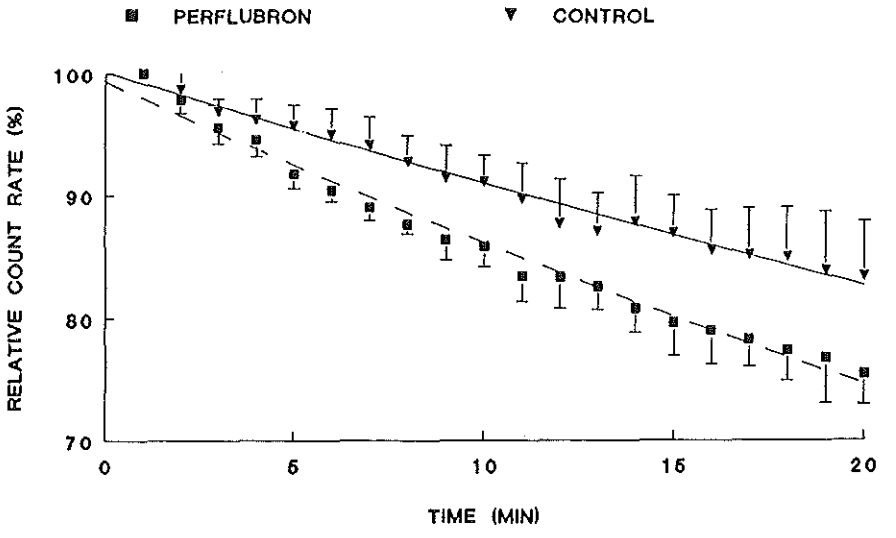

Figure 1. Time-activity curves of ^{99m}Tc-DTPA in the perfluorocarbon-treated (▲) and the control (■) groups. Values are given as mean ± SD.

and in response to increases in lung volume (i.e. high PEEP or large tidal volume ventilation) (23-25).

On intratracheal administration, perfluorocarbon distributes in the lung by acting on the alveolar air-liquid interface and by filling the alveoli, this depending on the volume administered as suggested in previous studies (6-8). After administration of perfluorocarbon (possessing a constant surface tension), the surface tension characteristics of surfactant at the alveolar air-liquid interface are altered (or governed by surface tension from perfluorocarbon) and a new film with a new surface tension is

established at the alveolar air-liquid interface due to evaporation of the perfluorocarbon (presumably higher than alveolar surfactant at end-expiration). This suggestion has been indirectly supported by a study on healthy animals in which arterial oxygenation remained unchanged when higher PEEP levels were applied with the same experimental ventilator settings (unpublished observations). It has been reported that changes in surface tension properties of the lung may be associated with changes in the permeability of alveolar epithelium (22,26). Thus, it may be speculated that the increased surface tension at the alveolar-air interface in the expiratory phase in perfluorocarbon-treated animals might have contributed to the increased clearance rate of ^{99m}Tc-DTPA in the healthy lungs.

The clearance of ^{99m}Tc-DTPA has been demonstrated to be a very sensitive indicator of alterations in pulmonary surfactant system (13,17,21). Evander and coworkers demonstrated that a detergent (dioctyl sodium sulfosuccinate), which acts only on the surfactant system and causes no structural damage to the alveolar epithelium (27), increases the clearance rate of ^{99m}Tc-DTPA without any observed changes in blood gases and lung mechanics (21). Bos et al. demonstrated that experimentally increased alveolar surfactant content reduces the clearance rate of ^{99m}Tc-DTPA in healthy animals, indicating the role for the surfactant system in limiting the alveolo-capillary transfer of solutes (13). The high sensitivity of clearance measurements of ^{99m}Tc-DTPA is an important point for consideration during interpretation of the present results. In one study, Bos and colleagues have shown an increased clearance rate of ^{99m}Tc-DTPA in healthy animals after introducing into the lungs a volume of saline as small as 5 ml (13): they suggested this was due to changes in lung surfactant function.

Therefore, one has to consider the possible interaction between the perfluorocarbon liquid and the lung surfactant in healthy animals. On the other hand, it is speculated that the hydrophobic and lipophobic perfluorocarbon liquid may impose another diffusion barrier to the hydrophilic tracer agent ^{99m}Tc-DTPA, which has an extremely low lipid solubility. However, if this were the case, we could expect to measure slower clearance rate in the perfluorocarbon group compared to the control group, as opposed to the present data.

The effects of increased lung volume on lung permeability in healthy animals have been studied during conventional gas ventilation. The clearance rate of ^{99m}Tc-DTPA remained at baseline value at a PEEP level of 2.5 cm H_2O, while increased PEEP levels above 5 cm H_2O increased the clearance rate in healthy animals (23). In another study in healthy animals, moderate levels of PEEP (2-10 cm H_2O) had little effect on clearance rate as compared to marked increases at PEEP level of 15 cm H_2O (28). Furthermore, high lung volumes - not airway pressure - increased the clearance rate of trace element (29). In the present study, we applied the least possible level of PEEP (2 cm H_2O) in order to prevent both the bulk movement of perfluorocarbon liquid along the airways and to eliminate the possible increases in lung volume that might arise from high PEEP levels. On the other hand, although the ventilation volumes were equal, there were only trivial differences in the insufflation pressures between the groups in this study. The increased peak airway pressure in the perfluorocarbon group was probably a reflection of high airway resistance during inspiration, since airway resistance was increased in this group following perfluorocarbon administration. Moreover, the end-inspiratory airway pressures, which might be taken as a reflection of transthoracic pressure, did not

significantly differ between the two groups during the study period. One might argue that the presence of a dense liquid (such as perfluorocarbon) in the lungs might act as an additional PEEP, causing further lung distension. Although there was no exact data on lung volume in the perfluorocarbon group the mean airway pressure, which may be taken as an estimate of lung expansion (volume), was slightly higher in the perfluorocarbon group compared to the control group. Based on the above discussion - whether this slight difference in pressure of approximately 1 cm H_2O between the mean airway pressures may have contributed to the increased clearance rate of the tracer remains to be further evaluated.

Regarding the above-mentioned discussion and based on our present experimental findings, we speculate that the increased permeability for small molecules in healthy animals may be due to the alterations in the surfactant system function after application of perfluorocarbon liquids. However, healthy animals treated with the same protocol could be restored to spontaneous breathing (after 3 h of partial liquid ventilation) without any changes in lung function by the seventh day after the study (unpublished observations). Furthermore, data from histological studies in healthy animals appeared to be normal within a few days after liquid ventilation (30), and remaining normal even after three years (31,32). Considering these points, it is likely that healthy animal lungs are capable of reversing any changes in permeability of the alveolo-capillary barrier, restoring normal lung function. Otherwise, ongoing changes would result in deterioration of pulmonary gas exchange and respiratory mechanics parameters. The ability of healthy lungs to overcome acute changes, as evaluated by pulmonary clearance measurements, has been demonstrated in human studies. Studies have shown

that symptom-free smokers have a faster ^{99m}Tc-DTPA clearance than non-smokers (33,34), and an increased clearance rate can be induced in non-smokers within 3 days of smoking (35) while it returns toward normal upon abstinence from cigarettes (36). On the other hand, it should be emphesized that the present results from healthy lungs implying increased permeability in the alveolo-capillary barrier may not be extrapolated directly to the injured lung, since the histological analysis of animal lungs after partial liquid ventilation has demonstrated decreased edema formation when compared to gas ventilated animals (8).

In conclusion, although the exact mechanisms responsible for the increased clearance rate of ^{99m}Tc-DTPA from alveoli to blood after partial liquid ventilation are not clear from the present results, we speculate that the effect of partial liquid ventilation on the pulmonary clearance of ^{99m}Tc-DTPA may reflect minimal reversible changes in the surfactant system in heathy lungs. Further investigations to evaluate the possible relationship between the increased pulmonary clearance of ^{99m}Tc-DTPA and lung surfactant properties are warranted, as well as the time and perfluorocarbon-dose effects on clearance of ^{99m}Tc-DTPA from healthy lungs with this new type of ventilatory support.

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SUMMARY AND CONCLUSIONS

Acute respiratory failure is a common clinical entity occurring in the presence of certain risk factors such as trauma, sepsis and pneumonia. Regardless of the pathogenic stimuli, hypoxia resulting from shunting of blood due to atelectasis and decreased pulmonary compliance due to increased alveolar surface forces are the main hallmarks of acute respiratory failure. The main therapeutic goal in such a clinical condition is to support the lungs for adequate oxygenation until the primary disorder is healed. There are various ventilatory support techniques for this purpose, one of which is liquid ventilation as outlined in Chapter 1.

Since the first introduction of PFC liquid in liquid breathing technique by Clark and Gollan in 1966, there has been continuous research in this area. PFC liquids offer unique respiratory medium with their extremely low surface tension and high ability for dissolving O_2 and CO_2. Liquid ventilation is performed by delivering tidal volumes of extracorporeally oxygenated PFC liquid with a modified liquid ventilator after filling the degassed lungs to functional residual capacity volume with PFC. It follows that the air-liquid interface in the PFC-filled alveoli is eliminated, thereby reducing the retractive forces along the alveolar lining due to the surface tension phenomenon. This point has made the liquid ventilation technique an alternative means of respiratory support. Various studies with liquid ventilation have shown this technique to provide adequate gas exchange and improve lung compliance, even in lung disease stages where other conventional supportive techniques were ineffective. This technique progressed to application in human preterm neonates suffering from severe respiratory failure (reported in 1990).

However, there has been limited clinical use of this technique due to the extra technical requirements.

Recently, the latest progress in this field was made by combining conventional gas mechanical ventilation with intratracheal PFC application, namely partial liquid ventilation (PLV). Chapter 1 summarizes the related literature on liquid ventilation and briefly describes the rationale behind PLV.

In order to investigate the efficacy of PLV as a ventilatory support means in acute respiratory failure, a study was designed to look for the dose-response effects on pulmonary parameters as described in Chapter 2. In this study, respiratory failure induced-animals were intratracheally treated with 3 ml/kg incremental doses of PFC up to 15 ml/kg while volume-controlled ventilation at conventional ventilator settings was superimposed. This study demonstrated for the first time that PLV improves pulmonary gas exchange in a dose-dependent manner in acute respiratory failure. Moreover, the improvement in respiratory mechanics, regardless of the PFC dose, revealed that PLV acts via different mechanisms for pulmonary parameters. That is, decreased surface tension at the alveolar air-liquid interface due to PFC holds for the improvements in respiratory system compliance and easy lung distension, while prevention of expiratory alveolar collapse by the space-occupying characteristic of PFC holds for the dose-dependent improvement in oxygenation.

In Chapter 3, PLV was compared to conventional mechanical ventilation in terms of respiratory parameters and lung histology using the same ventilatory parameters during a 3-h observation period in animals with acute respiratory failure. After intratracheal administration of PFC at a volume corresponding to functional residual

capacity (18 ml/kg), continuous positive pressure ventilation (CPPV) was superimposed while another group received no treatment but only CPPV. PaO_2 stayed around 60 mm Hg and $PaCO_2$ gradually increased over time in the CPPV group in contrast to increased PaO_2 (from 67±11 to 424±14 mm Hg) and well preserved $PaCO_2$ for several hours in the PLV group. Moreover, respiratory system mechanics were improved (airway pressures decreased, lung compliance increased) and cardiovascular parameters were not affected during PLV. On the other hand, post-mortem histological findings revealed that the alveolar structure was well preserved in the PLV group, while exudate and hyaline membrane formation were observed in the CPPV group. Considering the experimental data, PLV proved to be more effective than conventional mechanical ventilation by ensuring improved gas exchange at low inflation pressures.

To assess whether the changes achieved in gas exchange and respiratory mechanics in respiratory failure during PLV at the short-term could be of therapeutic benefit by maintaining these improvements for several hours, different PFC doses were tested in respiratory failure-induced animals in another study, presented in Chapter 4. The 6-h observational data confirmed the previous experimental results for oxygenation and lung mechanics. Furthermore, this study demonstrated a time-dependent characteristic among the different PFC doses in their ability to maintain the improvements in lung functions. That is, high PFC doses (9-12 ml/kg) reaching to functional residual capacity volume were required to maintain adequate gas exchange at low airway pressures for several hours. The relationship between the PFC dose and the time-dependent changes in lung functions suggested that evaporative losses are required to be replaced in order to extend the duration of beneficial effects. Additionally, this study

provided related data regarding the possible role of high inflation pressures for the risk of barotrauma during PLV.

Since PLV differs from liquid ventilation technique, PLV needs to be investigated with respect to its safety for normal lung function. Therefore, a study was designed to investigate the effects on gas exchange and lung mechanics in healthy adult animals and comparison to control values was made on the same animals one week after PLV trial for long-term changes. As described in Chapter 5, healthy animals tolerated 3 h of PLV with well maintained pulmonary gas exchange and lung mechanics parameters. Moreover, animals were successfully returned to spontaneous air breathing. The after-effects of PLV by the seventh day revealed that respiratory parameters (as assessed by PEEP titration) were similar to pre-PLV control values.

As a further detailed investigation of the effects of PLV on healthy lung function, a study was designed to evaluate the integrity of the alveolo-capillary barrier after PLV application in healthy animals since it is vital for normal lung function. For this study, measurement of pulmonary clearance of a radio-labelled tracer (technetium 99m diethylene triamine pentaacetate, ^{99m}Tc-DTPA), a very sensitive test for the integrity of the alveolo-capillary barrier, was performed after 3 h of PLV in healthy animals and compared with conventional mechanical ventilation (Chapter 6). The results demonstrated an increased pulmonary clearance rate of ^{99m}Tc-DTPA after PLV in comparison to conventional mechanical ventilation. Considering the normal gas exchange and lung mechanics data observed several days after PLV (in previous study), the increased clearance rate may reflect minimal reversible changes in the alveolo-capillary barrier in healthy animals.

The work presented in this thesis describes PLV as a new means of respiratory support. It is concluded that: 1-) PLV offers major advantages compared to liquid ventilation, such as ease of application without extra technical requirements; 2-) PLV is an effective technique in improving pulmonary gas exchange and respiratory mechanics in animals with respiratory failure and, in this regard, proves to be superior to conventional mechanical ventilation in the short-term; 3-) Healthy animals treated with PLV return to spontaneous breathing without any changes in lung functions at the long-term.

Considering the above documentation with favorable and encouraging results, it is likely that PLV will play an important role in future clinical practice. "Like the submarine and the spaceship, liquid breathing may soon undergo a vital transformation from science fiction to textbook."*

*Fuhrman BP. Perfluorocarbon liquids and respiratory support. Crit Care Med 1993; 21:951

SAMENVATTING EN CONCLUSIES

Acuut respiratoir falen is een klinisch beeld dat voorkomt in de aanwezigheid van bepaalde risico factoren, zoals trauma, sepsis en pneumonie. Hypoxie door het shunten van bloed a.g.v. atelectase én verminderde pulmonale compliantie door verhoogde oppervlakte spanning in de alveoli zijn de belangrijkste hoofdkenmerken, ongeacht het onderliggende lijden. Hoofddoel in een dergelijke klinische aandoening is het ondersteunen van de longfunctie ter bevordering van een adequate oxygenatie totdat de primaire aandoening is behandeld. Hiervoor zijn verschillende ondersteunende beademings technieken voorhanden, waarvan één vloeistofbeademing is **(Hoofdstuk 1)**.

Sinds de introductie van PFC vloeistof in de vloeistofbeademings techniek door Clark en Gollan in 1966, is er continu onderzoek op dit gebied. PFC biedt een uniek respiratoir medium met extreem lage oppervlakte spanning en een groot vermogen tot het oplossen van O_2 en CO_2. Vloeistofbeademing vindt plaats door het aanbieden van tidal volumes van extracorporaal geoxygeneerd PFC d.m.v. een gemodificeerde vloeistof ventilator. Dit gebeurt nadat de ontgaste longen gevuld zijn met een PFC volume gelijk aan de functionele residuale capaciteit. Het gevolg hiervan is dat de lucht-vloeistof overgang in de PFC gevulde alveoli is verdwenen, waardoor de retractieve krachten aan de binnenzijde van de alveoli, a.g.v. lage oppervlakte spanning van PFC, gereduceerd zijn. Hierdoor is vloeistofbeademing een alternatieve techniek voor respiratoire ondersteuning. Verschillende studies hebben aangetoond dat vloeistofbeademing een adequate gasuitwisseling en verbetering van de long compliantie bewerkstelligt, zelfs in longaandoeningen waarbij conventionele methoden niet effectief waren. De voortgaande

ontwikkeling heeft geleid tot toepassing van deze techniek bij humane prematuren met respiratoir falen (gepubliceerd in 1990). Echter door de extra technische benodigdheden is het klinische gebruik tot nu toe beperkt gebleven.

De laatste ontwikkeling op dit gebied is het combineren van conventionele mechanische beademing met intratracheale PFC toediening: partiële vloeistofbeademing (partial liquid ventilation, PLV). **Hoofdstuk 1** geeft een samenvatting van de relevante literatuur over vloeistofbeademing én geeft een korte beschrijving van de ratio achter PLV.

Met het oog op onderzoek naar de werkzaamheid van PLV als ventilatoire ondersteuning in acuut respiratoir falen, werd een studie ontworpen waarbij gekeken werd naar de dosis-respons effecten op long parameters (**Hoofdstuk 2**). Dieren met respiratoir falen werden behandeld met oplopende doseringen van 3 ml/kg tot 15 ml/kg intratracheaal toegediend PFC terwijl ze volume gecontroleerd beademd werden met conventionele ventilator instellingen. Deze studie toonde voor de eerste keer aan dat PLV op een dosis-afhankelijke wijze de pulmonale gasuitwisseling verbetert bij respiratoir falen. De verbetering van verschillende respiratoire parameters onafhankelijk van de PFC dosis, wijst erop dat PLV volgens verschillende mechanismen werkt. Te weten, de verlaagde oppervlakte spanning aan de alveolaire lucht-vloeistof overgang door PFC is verantwoordelijk voor verbetering van de compliantie in het respiratoire systeem en vergemakkelijkte ontplooiing van de longen. De dosis-afhankelijke verbetering van de oxygenatie wordt verklaard door preventie van eind-expiratoire alveolaire collapse door de ruimte innemende eigenschappen van PFC.

Hoofdstuk 3 beschrijft een studie waarbij PLV werd vergeleken met conventionele

mechanische beademing. Er werd gekeken naar respiratoire parameters en long histologie in dieren met acuut respiratoir falen, die gedurende 3 uur beademd werden met dezelfde ventilator instellingen. Eén groep werd na intratracheale toediening van PFC met een volume gelijk aan de functionele residuale capaciteit (18 ml/kg) beademd met continue positieve druk beademing (CPPV), terwijl de andere groep slechts beademd werd met CPPV zonder voorafgaande PFC toediening. In de CPPV groep bleef de PaO_2 rond 60 mmHg en nam de $PaCO_2$ geleidelijk toe, terwijl in de PLV groep de PaO_2 steeg (van 67 ± 11 naar 424 ± 14 mmHg) en de $PaCO_2$ gelijk bleef. Tevens waren de beademings parameters verbeterd (lagere luchtweg drukken, verbeterde long compliantie) en bleven de cardiovasculaire parameters ongestoord gedurende PLV. Aan de andere kant liet post-mortem long histologisch onderzoek zien dat de alveolaire structuur intakt was in de PLV groep, terwijl exsudaat en hyaline membranen gezien werden in de CPPV groep. Deze resultaten toonden dat PLV effectiever is dan conventionele mechanische beademing, daar een betere gasuitwisseling bereikt werd bij lagere inspiratie drukken.

Om te onderzoeken of de bereikte korte termijn veranderingen in gasuitwisseling en long mechanica gedurende PLV ook op lange termijn tot therapeutische verbetering kan leiden, werd de volgende studie ontworpen. In Hoofdstuk 4 werd het effect van verschillende PFC doseringen in dieren met acuut respiratoir falen gedurende een observatie periode van 6 uur onderzocht. De uitkomst van deze studie bevestigde de in eerdere hoofdstukken beschreven resultaten over oxygenatie en long mechanica. Verder toonde deze studie een tijd-afhankelijke factor aan in de handhaving van de verbeterde longfunctie na verschillende PFC doseringen. Hoge PFC doseringen (9-12

ml/kg) tot een volume gelijk aan de functionele residuale capaciteit waren nodig om adequate gasuitwisseling bij lage luchtweg drukken gedurende een aantal uren te handhaven. De relatie tussen de PFC dosering en het tijd-afhankelijke effect op de verbetering van de longfuncties, suggereert dat verlies van PFC door verdamping aangevuld moet worden om het gunstige effect te behouden. Tevens verschaft deze studie gegevens omtrent het mogelijke risico van hoge inspiratie drukken op het ontstaan van barotrauma tijdens PLV.

Daar PLV veschilt van de vloeistofbeademings techniek, moet de veiligheid van PLV op de normale longfunctie onderzocht worden. Om deze reden werd een studie in gezonde volwassen dieren opgezet om de effecten van PLV op de gasuitwisseling en long mechanica te onderzoeken. De resultaten hiervan werden 1 week later vergeleken met metingen bij dezelfde dieren om het lange-termijn effect te kunnen beoordelen. Zoals beschreven in **Hoofdstuk 5** bleven gasuitwisseling en long mechanische parameters onveranderd gedurende 3 uur PLV. De dieren waren instaat om na 3 uur weer spontaan op lucht te ademen. Na één week bleek dat de beademings parameters (verkregen door PEEP titratie) gelijk waren aan controle waarden vóór PLV.

Om de effecten op de gezonde longfunctie verder te bestuderen, werd een studie ontworpen om de invloed van PLV op de integriteit van de alveolaire-capillaire barrière te onderzoeken. Voor het doel van deze studie werden metingen van de pulmonale klaring van radio-actief gelabelde technetium-99m-diethylene triamine pentaacetaat (^{99m}Tc-DTPA), een zeer gevoelige test voor de integriteit van de alveolaire-capillaire barrière, uitgevoerd in gezonde dieren na 3 uur PLV óf conventionele mechanische beademing **(Hoofdstuk 6)**. De resultaten van deze studie toonden een verhoogde pulmonale klaring

van ^{99m}Tc-DTPA na PLV in vergelijking tot conventionele mechanische beademing. De normale gasuitwisseling en long mechanica 1 week na PLV in beschouwing nemend (vorige studie), kan de verhoogde klaring een afspiegeling zijn van minimale reversibele veranderingen van de alveolaire-capillaire barrière in gezonde dieren.

In dit proefschrift wordt PLV als een nieuwe methode voor respiratoire ondersteuning beschreven. Er wordt geconcludeerd dat: 1-) PLV grote voordelen biedt t.o.v. vloeistofbeademing, zoals gemak van toepassing zonder extra technische benodigdheden; 2-) PLV een effectieve techniek is om gasuitwisseling en long mechanica in dieren met respiratoir falen te verbeteren en, in dit opzicht, superieur is t.o.v. conventionele mechanische beademing op korte termijn; 3-) gezonde dieren behandeld met PLV kunnen overgaan tot spontaan ademen zonder veranderingen in longfunctie op lange termijn.

Bovenstaande gunstige en veelbelovende resultaten in beschouwing nemend, is het aannemelijk dat PLV in de toekomst in de kliniek een belangrijke rol zal spelen. "Zoals de onderzeeboot en het ruimteschip, zal vloeistof ademhaling binnenkort een belangrijke overgang van science fiction naar tekstboek ondergaan."*

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CURRICULUM VITAE

Ahmet Salih Tütüncü was born on 30th of December, 1961, in Konya, Turkey. He got high school degree from Konya Anadolu High School in 1980 and, the same year, he attended the Medical Faculty of University of Istanbul as a medical student. He received the diploma of Medical Doctor on 31st of July, 1986. After medical training, he worked as a general practitioner at a state hospital for one year. In 1987 he started anesthesia training at the Department of Anesthesiology and Intensive Care in the University of Istanbul. From 1990 till 1992 he worked as a research fellow at the Department of Anesthesiology in Erasmus University Rotterdam, The Netherlands. He got his diploma of Anesthesia and Intensive Care from the University of Istanbul on 31st of July, 1992. At the moment he is working as a staff member in the Intensive Care Unit of the Department of Anesthesiology at the Medical Faculty of University of Istanbul.

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