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Experimental study of partial liquid ventilation in the setting of acute respiratory failure induced by sea water lung lavage in rabbits.

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Experimental study of partial liquid ventilation in the setting of acute respiratory failure induced by sea water lung lavage in rabbits.*

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Abstract

To study the effect of partial liquid ventilation (PLV) with perfluorocarbon on acute respiratory failure, 3 groups of 17 rabbits were examined to compare. After acute respiratory failure was induced by lung lavage with sea water in 12 of the 17 rabbits, 7 of the 12 rabbits were treated with conventional mechanical ventilation (AC group) and 5 of the 12 rabbits were treated with PLV using perfluorocarbon (AP group). The remaining 5 normal rabbits without acute respiratory failure were treated with PLV with perfluorocarbon as a control group (PL group). In the PL group, PaO2, PaCO2, blood pH, pulmonary compliance or pathological findings were not so changed after PLV. In the AC and AP groups, PaCO2 significantly increased, and in contrast, PaO2 and pulmonary compliance significantly decreased after lung lavage. However, these findings improved to almost the same levels as those of a control group within 2 h after the PLV treatment in the AP group, but in the AC group, these gradually deteriorated over time. As for the pathological findings, pulmonary vascular congestion, alveolar hemorrhage and inflammatory infiltration were observed in the AC group. However, these findings were not observed in the specimens of the AP group. From these results, PLV with perfluorocarbon was shown to be useful to improve gas exchange and pulmonary functions without major side effects.

KEYWORDS: liquid ventitation, perfluorocarbon, acute respiratory failure, lung lavage, sea water

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Experimental Study of Partial Liquid Ventilation in the Setting of Acute Respiratory Failure Induced by Sea Water Lung Lavage in Rabbits

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To study the effect of partial liquid ventilation (PLV) with perfluorocarbon on acute respiratory failure, 3 groups of 17 rabbits were examined to compare. After acute respiratory failure was induced by lung lavage with sea water in 12 of the 17 rabbits, 7 of the 12 rabbits were treated with conventional mechanical ventilation (AC group) and 5 of the 12 rabbits were treated with PLV using perfluorocarbon (AP group). The remaining 5 normal rabbits without acute respiratory failure were treated with PLV with perfluorocarbon as a control group (PL group). In the PL group, PaO₂, PaCO₂, blood pH, pulmonary compliance or pathological findings were not so changed after PLV. In the AC and AP groups, PaCO₂ significantly increased, and in contrast, PaO₂ and pulmonary compliance significantly decreased after lung lavage. However, these findings improved to almost the same levels as those of a control group within 2h after the PLV treatment in the AP group, but in the AC group, these gradually deteriorated over time. As for the pathological findings, pulmonary vascular congestion, alveolar hemorrhage and inflammatory infiltration were observed in the AC group. However, these findings were not observed in the specimens of the AP group. From these results, PLV with perfluorocarbon was shown to be useful to improve gas exchange and pulmonary functions without major side effects.

Key words: liquid ventilation, perfluorocarbon, acute respiratory failure, lung lavage, sea water

V arious interventions, including positive end expiratory pressure, extracorporeal life support, inverse ratio ventilation and high frequency ventilation have been used to improve pulmonary function and gas exchange in patients with severe acute respiratory failure. However, mortality rate in such patients is still high. One of the reasons for this is that conventional mechanical ventilation does not improve pulmonary shunt. Furthermore, prolonged mechanical ventilation induces several complications, such as barotrauma, volutrauma, oxygen toxicity and pneumonia (1-3).

In 1966, Clark and Gollan first reported that mice and cats could survive in a fluorocarbon liquid for several hours. They presumed that fluorocarbon liquid might have used in the medical field (4). Since that time, many studies of liquid ventilation have been done in the United States and Europe, and most of them have reported the efficiency of liquid ventilation with perfluorocarbon in a respiratory failure model in animals (5, 6). Recently Phase I and II studies showing the efficiency of liquid ventilation in respiratory failure have been reported in the United States (7–9). We have also started animal experiments on partial liquid ventilation (PLV).

The purpose of this study is to evaluate the effects of PLV with perfluorocarbon (perfluorooctyl bromide: the Green Cross Corporation, Osaka, Japan) on this acute respiratory failure model.

Materials and Methods

Adult New Zealand White rabbits obtained from

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Table I Properties of perfluorocarbon (Perfluorooctyl bromide)

Purity by gas chromatography	More than 99.9%
Solubility in water	Practically insoluble
Density d20	1.934
Boiling point	4 - 42°C
Vapor pressure at 37°C	II.2mmHg
Oxygen solubility at 37°C	41.6 vol%
Carbon dioxide solubility at 37 °C	198.5 vol%

Shimizu Experimental Supply Inc., (Kyoto, Japan), weighing 2.7-3.4 kg were anesthetized with intravenous injection of thiamylal sodium $(0.05 \,\mathrm{mg/kg})$ through an auricular vein after intramuscular injection of ketamine hydrochloride (50 mg/kg) and atropine sulfate (0.5 mg/)body). Rabbits were placed in a supine position. Tracheotomy was performed and intubated with an endotracheal tube. Mechanical ventilation (No. 15-786, Shinano Industry, Tokyo, Japan) was initiated. The ventilator was set at FiO_2 1.0, tidal volume 10 ml/kg, respiratory rate 25/min, inspiratory/expiratory ratio 1:1, positive endexpiratory pressure (PEEP) $5 \text{ cm } H_2O$. These settings were maintained throughout the experiment. Anesthesia was maintained with continuous infusion of pentobarbital sodium (1 mg/h) through an auricular vein. Drip infusion of saline was given at 2 ml/h via an auricular vein. Vecuronium bromide (0.4 mg) was injected as required for

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muscle paralysis. No other infusions or drugs were given. An internal carotid artery was cannulated with a 14 G catheter for arterial pressure monitoring and blood sampling. Airway pressure was monitored at the distal site of the endotracheal tube.

After baseline measurements, the 17 rabbits were divided into three groups. PLV was performed on one group of rabbits with normal, non-respiratory failure lungs as a control; this group was called the PL group. The method of PLV is as follows: A dose of 15 ml/kg of perfluorocarbon was administrated into the lungs slowly through a side port of the airway circuit over 3-5 min. Mechanical ventilation was continued during administration. In the other two groups, sea water lung lavage was performed to induce acute respiratory failure. Lung lavage with 10 ml/kg of sea water was performed repeatedly to achieve PaO₂ under 100 mmHg. After each lavage, sea water was suctioned through the endotracheal tube. Most of the sea water (90-95%) could be drained finally. PaO₂ under 100 mmHg was achieved by three or four repetitions of lung lavage. After the lavage, one group was treated with PLV (acute respiratory failure + PLV; AP group), and the other group was treated with conventional mechanical ventilation (acute respiratory failure + conventional mechanical ventilation; AC group) (Fig. 1).

 PaO_2 , $PaCO_2$, pH and pulmonary compliance, given as the tidal volume divided by peak airway pressure, were



Fig. I Protocols of the experiment. PL: Normal, non-respiratory failure lung group treated with partial liquid ventilation (PLV) (n = 5); AC: Acute respiratory failure (ARF) group treated with conventional mechanical ventilation (CMV) (n = 5); AP: Acute respiratory failure group treated with PLV (n = 7).

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assessed every 15 min for 2h (Sannei Polygraph 360 system, Nippon Electric Company, Tokyo, Japan).

After 2h, all animals were sacrificed and the heartlung blocks were excised. In the AP and AC groups, perfluorocarbon in lungs was drained. The lungs were placed in a 10 % formaldehyde solution. Hematoxylineosin staining was performed for histopathological evaluation.

All data are presented as mean \pm standard deviation. Analysis of variance (ANOVA) was used for statistical analysis to compare each group. $P \leq 0.001$ was consid-

> 700 600

PaO₂(mmHg)

ered to be significant.

Results

Sea water lung lavage induced acute respiratory failure in all rabbits of the AC and AP groups, as demonstrated by significant decreases in PaO_2 and pulmonary compliance, significant increase in $PaCO_2$. All rabbits survived during the experiment. Pneumothorax or perfluorothorax was not observed at autopsy.

 PaO_2 . PaO₂ was over 500 mmHg at the baseline





Fig. 3 PaCO₂ in the three groups. - ; - - ; - ; *: See legend to Fig. 2.

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in all the groups without a statistical difference. In the PL group, PaO₂ decreased to approximately 400 mmHg after initiation of PLV, and was sustained approximately the same level throughout the experiment. In the AC and AP groups, PaO₂ deteriorated to under 100 mmHg just after sea water lavage which indicates severe acute respiratory failure. PaO₂ deteriorated continuously after sea water lavage in the AC group. In contrast, PaO₂ improved significantly to over 200 mmHg just after initiation of PLV and was sustained throughout the experiment in the AP group (P < 0.001) (Fig. 2).

 $PaCO_2$. PaCO₂ increased to approximately 45 mmHg after initiation of PLV in the PL group, but it gradually returned to its previous level. PaCO₂ increased just after lung lavage in the AC and AP groups. In the AC group, PaCO₂ deteriorated immediately after lung lavage and continued to deteriorate over time (P < 0.001). In the AP group, however, it improved gradually to 46 mmHg, which was approximately the same value as in the PL group (P < 0.001) (Fig. 3).

pH. Blood pH value of PLV in the PL group was maintained within the normal range, while that in the AP



Fig. 4 pH in the three groups. — **(**, - **(**, -, **(**,



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Fig. 6 Light microscopic photographs of rabbit lungs. PL: Normal, non-respiratory failure lung group treated with partial liquid ventilation (PLV); AC: Acute respiratory failure group treated with conventional mechanical ventilation; AP: Acute respiratory failure group treated with PLV (Hematoxylin eosin, \times 200).

group deteriorated briefly before stabilizing the same value as that in the PL group after initiation of PLV. In contrast, pH showed a gradual deterioration in the AC group after sea water lavage (P < 0.001) (Fig. 4).

Pulmonary compliance. After initiation of PLV, pulmonary compliance in the PL group decreased a little but did not decrease further throughout the experiment. Pulmonary compliance deteriorated after lavage in the AP and AC groups. But compliance improved to approximately the same value as that of the PL group immediately after the administration of perfluorocarbon in the AP group (Fig. 5).

Light microscopic assessment. Light microscopic assessment did not reveal destruction of lung structures, alveolar hemorrhage or inflammatory infiltration in the PL group. Pulmonary vascular congestion, alveolar hemorrhage and inflammatory infiltration were observed in the AC group, all consistent with adult respiratory distress syndrome (ARDS). However, the AP group specimens showed a reduction in severity of these findings (Fig. 6).

Discussion

Liquid ventilation has been investigated in the United

States and Europe over the last three decades (11, 12). Many reports have shown the efficacy of liquid ventilation (13, 14). Clinical phase I and II studies are now going on. However, there are still many subjects to be studied including the mode of ventilation and influence on inflammatory cascades.

In this study, sea water lung lavage induced severe acute respiratory failure with pulmonary vascular congestion, alveolar hemorrhage and inflammatory infiltration, all consistent with ARDS. Saline lung lavage is a common method to create a respiratory failure model. However, normal saline may wash out only alveolar surfactant and may not yield a respiratory failure model which is close to ARDS. On the other hand, because sea water is more hypertonic than normal saline solution, sea water lung lavage is thought to create more damage in lungs. In addition, with using sea water lavage, our laboratory has been able to effect easily reproducible and stable respiratory failure in dogs, without hemodynamic depression as exist in the oleic acid lung injury model (15, 16).

In PLV, lungs are filled with a volume of perfluorocarbon equivalent to the functional residual volume. Functional residual volume is different among species and also different among individuals. It was difficult to decide exactly how much the functional residual volume in rabbits

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was. In our experiment, the functional residual volume was decided when a meniscus of fluid was consistently visible in the endotracheal tube during momentary disconnection from the ventilator (17). The functional residual volume was approximately 15 ml/kg in rabbits. And when a volume of more than $15 \,\mathrm{ml/kg}$ was administrated, the liquid flew backward into the endotracheal tube during expiration, and high airway pressure was needed to push the liquid which overflowed into the endotracheal tube during expiration into the peripheral lung during the inspiratory phase. This high airway pressure was observed as a spike of airway pressure at the beginning of inspiratory phase. Therefore, when a spike of increase of airway pressure at the beginning of inspiration was observed, the volume of administrated perfluorocarbon was thought to be too much (18).

Hypoxemia, deteriorated pulmonary compliance and inflammatory infiltration improved in our experiment. Possible mechanisms by which PLV improves gas exchanges, pulmonary mechanics and pathological findings could include re-expansion of alveoli, due to the low viscosity and high-density properties of perfluorocarbon (19, 20). This improves intrapulmonary shunting. PLV may also act as an alternative respiratory medium in surfactant-deficient lungs due to the low surface tension properties of perfluorocarbon. PLV improved pulmonary compliance. In addition, perfluorocarbon can dissolve large volumes of respiratory gases. Because of these effects, partial liquid ventilation with perfluorocarbon was thought to improve pulmonary mechanics and pulmonary gas exchange in this model (6, 7, 12, 15, 16).

Perfluorocarbon is thought to be nontoxic to the human body (11, 21). Major side effects were not observed and lung specimens did not demonstrate any specific drawbacks due to perfluorocarbon in our experiment. However, our experiment was just for 2h. In a clinical setting, PLV may have to be continued for up to several days. Prolonged PLV could have detrimental effects on the lungs. Forman *et al.* reported on a fine structure study on the lungs of newborn rabbits in which ventilation with perfluorocarbon was revealed to damage the ultrastructure of the lungs (22). Modell *et al.* also reported that perfluorocarbon remains for as long as 3 years in the lungs of primates (23). Thus, the effects of prolonged PLV on mammalian lungs are not yet fully understood.

Michele *et al.* reported that perfluorocarbon might have an anti-inflammation effect (24). It is also reported that macrophages phagocytose perfluorocarbon droplets.

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Engulfed perfluorocarbon may have an influence on the release of chemical mediators of macrophages and this may contribute to an anti-inflammation.

Thus there are still many aspects of PLV and perfluorocarbon to be studied as mentioned above.

In conclusion, PLV with perfluorocarbon significantly improved gas exchange and pulmonary mechanics in our model of acute respiratory failure without major side effects.

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