Effect of partial liquid ventilation and nebulized perfluorocarbon on CT lung density distribution: randomized controlled study of experimental lung injury

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Background. Perfluorocarbon (PFC) liquid can improve gas exchange in acute lung injury. How PFC aerosol is distributed in the lung is unknown.

Methods. We induced lung injury in rabbits with saline lavage, followed by mechanical ventilation in the supine position. The animals were divided into three groups: a control group, a group treated with partial liquid ventilation and a group given nebulized perfluorocarbon (PF 5080). We made CT image slices of the excised lungs. In the apical, middle and caudal slices we defined three regions of interest, from anterior to posterior, and noted the mean attenuation of each area. We also studied two rabbits which had not received lung injury or mechanical ventilation.

Results. Group means were different between the normal rabbits and all three study groups. There was a difference between the control and partial liquid ventilation groups, and between the partial liquid ventilation and nebulized groups, but no difference between the nebulized and control groups. Within each treatment group, there was no regional difference in the distribution of density.

Conclusions. PF 5080 is not deposited in large amounts by aerosol. Less PFC was found in the lungs after partial liquid ventilation than expected. Within treatment groups, lung densities indicate less gravitational and regional differences than found in other studies.

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Partial liquid ventilation with perfluorocarbon (PFC) is a promising novel means of respiratory support in acute lung injury (ALI). PFCs are dense and may enter the dependent regions of the lung, distending collapsed lung by acting as ‘liquid PEEP’. CT scanning is used to assess and investigate the lungs in adult respiratory distress syndrome (ARDS), but rarely to study the effects and distribution of PFC during liquid ventilation in experimental ARDS.

In partial liquid ventilation, PFC is usually poured into the airway. Aerosols are used to introduce drugs into the respiratory tract, but PFCs have rarely been given in this way. PFC has been given by jet nebulizer into the endotracheal tube, with promising results. Until the present study, CT had not been used to study nebulized PFC used for treatment of lung injury.

Methods and results

We studied 26 young adult female New Zealand white rabbits (10 control rabbits, six treated with PLV and 10 treated with nebulized PFC). Procedures were conducted according to the UK Animal Act (Home Office Project Licence PPL 60/2181). We induced and maintained anaesthesia with urethane, and neuromuscular blockade with pancuronium. We placed a 4 French gauge catheter in a carotid artery and another 4 French gauge catheter in an internal jugular vein,
using methods reported previously. The animals were supine. After tracheostomy, the lungs were ventilated with an inspired oxygen fraction \( F_{\text{io}} \) of 1.0 and inspiratory duration of 1 s using an SLE 250 paediatric pressure-controlled time-cycled ventilator (Specialised Laboratory Equipment, Croydon, Surrey, UK). The ventilator was first set with a pressure limit to obtain a tidal volume of 10 ml kg\(^{-1}\), measured with a Ventrak 1550 Respiratory Mechanics Monitoring System (Novametrix Medical Systems, Wallingford, CT, USA), a respiratory rate of 30 bpm and 4 cm H\(_2\)O PEEP.

We induced lung injury by repeated lavage with warmed 0.9% saline until the arterial oxygen partial pressure \((P_{\text{ao}})\) was <13.3 kPa in two arterial blood samples taken 15 min apart, with a peak inspiratory pressure of 24 cm H\(_2\)O and 4 cm H\(_2\)O PEEP.

Animals were allocated randomly to one of the following treatment groups.

1. Control (10 animals).
2. Partial liquid ventilation (PLV) (six animals). Warmed PFC PF 5080 (3 M Chemicals, Bracknell, Berks, UK), was poured into the endotracheal tube (20 ml kg\(^{-1}\), or until a meniscus was seen in the endotracheal tube at the level of the sternum when PEEP was temporarily switched off). Further PF 5080 was added through a side arm of the endotracheal tube so that the meniscus remained at this level, checked intermittently at zero PEEP.
3. ‘Nebulized PFC’ (10 animals). We administered 20 ml kg\(^{-1}\) PF 5080 using an ultrasonic nebulizer (Devilbiss Ultra-Neb 2000, Sunrise Medical, UK) set at maximum output (1.63 MHz). We estimated the hourly requirement to be 13 ml kg\(^{-1}\) h\(^{-1}\) according to the formula used by Salman and colleagues.

After randomization we ventilated the animals’ lungs with an inspiratory tidal volume of 10 ml kg\(^{-1}\) (maximum inspiratory pressure 45 cm H\(_2\)O) for 15 min before starting treatment. We checked respiratory, cardiovascular and gas exchange values hourly as previously described. After 12 h, surviving animals were killed with an injection of urethane. After death we clamped the lungs at end-expiration whilst maintaining 4 cm H\(_2\)O PEEP, removed the lungs en bloc, immersed them in liquid nitrogen and stored them at \(-70^\circ\)C until CT scanning. For comparison, the lungs of two healthy rabbits which had not received lung injury or mechanical ventilation were also excised, frozen and scanned.

We used an IGE Medical Systems Hi-Speed Advantage Rapid Processing CT Scanner (IGE Medical Systems, Milwaukee, USA) with the following settings: 100 kV; 100 mA; pixel size 0.64×0.64 mm; 1 mm slice thickness (thus the voxel size was 0.64×0.64×1 mm); 5 mm gaps; 1 s exposure with a bone algorithm (high resolution) axial acquisition. The lungs were placed in crushed ice and scanned after blinding to treatment group. Three transverse images from cranial to caudal were made for each lung to give an apical, middle and caudal slice. In each slice we defined an anterior, middle and posterior region of interest (ROI) that consisted of lung parenchyma and did not include large vessels or airways (Fig. 1). The mean attenuation (Hounsfield units [HU]) was calculated for each ROI. We also measured the attenuation of pure PF 5080 with the same CT settings. One control group specimen and one nebulized group specimen were unsuitable for measurement because of fractures.

The groups were compared using the Kruskal–Wallis test, with Dunn’s correction for multiple comparison. Within-group comparisons for each lung area were also made for the three study groups. Suitable samples were obtained from nine control, six PLV, nine nebulized and two normal animals, with CT densities measured in nine fields in each animal.

Data are shown in Table 1. There was a highly statistically significant difference in CT densities between control and PLV and between control and normal rabbits \((P<0.001)\). Comparisons between PLV and nebulized groups, and between PLV and normal groups, were also significant \((P<0.001)\). There was a difference between the nebulized and normal groups \((P<0.001)\), but not between the nebulized and control groups.

We found no difference in the distribution of densities within groups, in either the craniocaudal or anterioposterior direction, for any of the three study groups.

### Discussion

As expected, the injured lungs were denser than those from healthy animals. Lung density after nebulized PFC was similar to the lung density in the control group, indicating that nebulization deposited little PFC in the lungs. We have
shown that nebulized PFC does not affect lung mechanics or gas exchange. In contrast, using an intratracheal jet nebulizer to give FC 77 (which has similar physical properties to PF 5080) improved oxygenation substantially in piglets with lavage injury. PFC is probably better deposited in the lungs by intratracheal administration. Our continuous-flow ventilator had the nebulizer in the circuit and the inspired gas was a small percentage of the total circuit flow, so that much of the nebulized PFC would not have been in the inspired gas. When PFC vapour was given with a rebreathing circuit, gas exchange was improved.

The lungs of animals treated with instilled PLV were denser than the lungs of animals in other groups. These animals had a substantial sustained improvement in oxygenation compared with control animals and those given nebulized PFC. PFC will increase tissue density, but the increase was less than expected from the density of PF 5080. Radiographic density reflects all the tissue components and is affected by aeration and by interstitial and alveolar fluid, and so we cannot estimate exactly how much PFC was in the lung. The CT density of pure PF 5080 is 606 HU. The radiographic density is determined by both physical density and electron density and differs slightly from the physical density which is 1.76 g ml\(^{-1}\) for PF 5080. An approximation value of the physical density of a substance can be calculated from the radiographic density using the following formula:

\[
\text{CT number} = K \left( \frac{\mu - \mu_w}{\mu_w} \right)
\]

where \(K\) is a constant (usually 1000), \(\mu\) is the linear attenuation coefficient of the sample and \(\mu_w\) is the linear attenuation coefficient of water. For PF 5080 with a density of 606 and a density of water of 1, the formula yields

\[
\mu = 606/1000 + 1 = 1.6.
\]

For a median density of 8, the lung tissue could consist mainly of oedema fluid alone or 37% air and 63% PF 5080. A small amount of material with high attenuation such as PF 5080 would substantially increase the density of the measured tissue. We found values in lungs treated with PLV that were not much greater than those in the untreated lungs, suggesting that either less PFC is present than expected or that more space is occupied by low attenuation material such as gas or fluid. Studying the effects of PEEP added to PLV, Kaisers and colleagues found a combination of PFC with oedema and lavage fluid in the airways. Airway obstruction would prevent PF 5080 from moving into the alveoli. Lung tissue beyond this obstruction would have the radiographic density of collapsed lung (~1 HU) rather than that of PF 5080. Another reason why alveoli may not completely fill with PFC is because gas is trapped in them at end-expiration. The exact distribution of gas in the alveoli is unclear. The trachea was clamped in end-expiration to maintain PEEP, but the lungs had been filled to functional residual capacity with PF 5080, which may explain some gas trapping.

Other CT studies of ARDS and PLV found that lung density varied with gravity. These studies were of adults with ARDS. We did not find gravity-related differences, but in small animals the weight of the overlying lung is less likely to cause collapse because the thorax is smaller than in larger animals or humans.

Studies using CT scanning on lung-injured animals receiving PLV have been performed in vivo. Practical and financial reasons prevented us from scanning the lungs in vivo, and so we froze them in liquid nitrogen and scanned them later. This may introduce an artefact. Freezing caused fissuring of some of the lungs and damaged two specimens so much that they were unsuitable for examination.

In summary, lung density in control animals and animals receiving nebulized PF 5080 was similar, and so this appears to be an ineffective method for PFC administration. CT attenuation was greater in animals treated with PLV than in control animals and animals treated with nebulized PFC, but was less than predicted for the alveoli being completely filled with PFC.

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References