

## Partial Liquid Ventilation with Perfluorocarbon in the Treatment of Rats with Lethal Pneumococcal Pneumonia

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**Background:** Partial liquid ventilation with perfluorocarbon is a new therapeutic strategy to treat various lung disorders. The current study was undertaken to determine the efficacy of partial liquid ventilation with a perfluorocarbon (FC-77) in the treatment of pneumococcal pneumonia in rats.

**Methods:** Male Wistar rats (weight, 275–300 g; n, 75) were infected *via* direct intratracheal inoculation with ca  $10^9$  colony-forming units of viable *Streptococcus pneumoniae*, serotype 3, and 24 h after infection were placed into one of five groups, each containing 15 rats. The groups were (1) no treatment, (2) one intramuscular injection of penicillin G benzathine (200,000 U), (3) partial liquid ventilation with FC-77, (4) partial liquid ventilation with FC-77 and a single intramuscular dose of penicillin G benzathine (200,000 U), and (5) gas ventilation. Animals were observed every 24 h for survival.

**Results:** All untreated or gas-ventilated animals or animals that received only partial liquid ventilation were dead by 7 days. Those receiving only partial liquid ventilation survived longer than untreated controls, but ultimately all succumbed by day 7. Survival was 40% for penicillin-treated rats compared with controls ( $P < 0.05$ ) and 80% for animals treated with both partial liquid ventilation and penicillin *versus* antibiotic alone ( $P < 0.05$ ).

**Conclusions:** These observations suggest that partial liquid ventilation with perfluorocarbon in combination with antibiotic administration may be an effective therapeutic modality in pneumococcal pneumonia. (Key words: Antibiotic therapy; artificial respiration; inflammation; *Streptococcus pneumoniae*; surface-active agents.)

PARTIAL liquid ventilation with perfluorocarbons or perfluorocarbon-associated gas exchange (PAGE) is a technique in which the functional residual capacity is filled with a perfluorocarbon and the lungs are mechanically ventilated in a traditional fashion.<sup>1</sup> Use of PAGE or full liquid ventilation in animal models of surfactant deficiency,<sup>2–5</sup> gastric acid aspiration,<sup>6</sup> meconium aspiration,<sup>7</sup> pneumonia,<sup>8</sup> congenital diaphragmatic hernia,<sup>9</sup> and acute respiratory distress syndrome<sup>10,11</sup> has resulted in improvements in oxygenation, lung compliance, histopathology, and survival. Perflubron, the only medical-grade perfluorocarbon available, has been shown to have some antiinflammatory properties *in vitro* and *in vivo*.<sup>12–14</sup> Therefore, the use of PAGE in the acutely infected and inflamed lung may provide a benefit that is not limited to its immediate effect on surface tension, compliance, and oxygenation.

The therapy for severe community-acquired pneumonia includes the administration of systemic antibiotic agents and, at times, hospitalization with tracheal intubation and mechanical ventilation. This approach is usually successful; however, in some patients with an extensive pulmonary inflammatory response, a fatal outcome is noted. In pneumonia caused by pyogenic bacteria, the inflammatory response leads to filling of alveolar air spaces with proteinaceous and cellular debris. In the most severe cases, the inflammatory response progresses despite initiation of antibiotic therapy and death may result. One possible treatment strategy in such situations includes therapy directed at both the microbiologic and inflammatory components of the

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INOCULATION WITH *STREPTOCOCCUS PNEUMONIAE*

THERAPY AT 24 HOURS

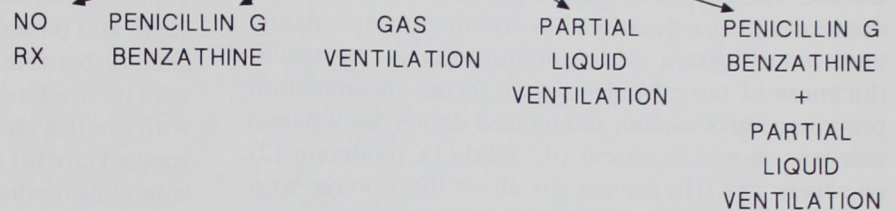


Fig. 1. Diagram of the experimental protocol.

disease process. The purpose of this investigation was to test the hypothesis that PAGE with the perfluorocarbon FC-77, in combination with penicillin, would lead to reduced mortality in rats with pneumococcal pneumonia.

## Methods

### Experimental Methods

All methods were approved by the University of Massachusetts Medical Center Animal Research Committee and are in accordance with the National Institutes of Health guidelines for animal use.

*Streptococcus pneumoniae*, serotype 3 (ATCC 6303), was propagated on 5% sheep blood agar incubated at 37°C in 5% CO<sub>2</sub> for 24 h. Immediately before infection of rats, bacteria were harvested from culture plates and suspended at a concentration of ca 10<sup>9</sup> colony-forming units/ml in sterile 0.85% NaCl containing 5% sucrose. Seventy-five antigen-free male Wistar rats (Charles River Laboratories, Portage, WI) weighing 275–300 g were maintained with free access to food and water for 2 weeks before infection to ensure the health of the experimental animals. At the time of infection, the rats were anesthetized with 2–3% halothane in oxygen. A 20-gauge catheter was placed into the trachea *via* direct laryngoscopy, and 1 ml of bacterial suspension was instilled directly through the catheter. Animals were allowed to recover briefly in 100% oxygen, returned to their quarters, and allowed free access to food and water.

Animals were divided into five groups of 15 each, and all animals were inoculated with *Streptococcus pneumoniae* (fig. 1). All treatments were instituted 24 h after inoculation. Treatments were neither randomized nor blinded. Controls received no treatment. Gas-ventilated animals were anesthetized with 2.5% halothane in oxygen and intubated *via* direct laryngoscopy with a 20-gauge catheter. A rodent pump (Harvard Apparatus, Inc., South Natick, MA) was used for ventilation and was set at a respiratory frequency of 120 breaths per minute and tidal volume of 5 ml. Ventilation time was 10 min. Animals who got partial liquid ventilation were intubated in the same fashion as the gas-ventilated animals. Fifteen milliliters of preoxygenated FC-77 (3M, St. Paul, MN) were instilled directly into the lungs followed by mechanical ventilation as described for the gas-ventilated animals. Any excess FC-77 above the functional residual capacity was exhaled around the uncuffed, loosely fitting endotracheal tube. Penicillin-treated animals received a single intramuscular dose of 200,000 U of penicillin G benzathine (Wyeth Laboratories, Philadelphia, PA). The last group (combined treatment) received a single intramuscular dose of penicillin G benzathine and partial liquid ventilation as described previously. Anesthetized animals were recovered in 100% oxygen for 1 h after treatment. The rats were observed every 24 h for survival for a total of 10 days.

### Characterization of Infection

In preliminary experiments, five infected but untreated controls were killed *via* exsanguination at one of three times (*i.e.*, 4, 12, and 24 h), and the heart and



lungs were removed *en bloc*. The lungs were inflated and placed in a 10% buffered formalin solution for preservation. Vertical sections were taken, fixed in paraffin, cut on a microtome, and stained with hematoxylin and eosin. The following system, which incorporated seven pathologic (two gross and five microscopic) criteria for acute lung disease, was used to define the extent of disease. The two gross pathologic parameters were induration and discoloration. The five microscopic parameters were extent of nonhomogeneous expansion, thickness of interalveolar septae, acute inflammation, proteinaceous exudate, and luminal debris. Each parameter was scored as absent (0), mild (1), moderate (2), or severe (3). The scores for all of the criteria were summed. Thus, a healthy lung would have a score of 0, and the score for an acutely injured lung could range from 1–21. One of us (AF), familiar with the scoring system but blinded to the time when the animals were killed, scored the gross and histologic specimens.

Four, 12, and 24 h after infection, animals were selected randomly for blood culture analysis. The tail of the rat was prepared with 10% povidone-iodine solution and snipped 1 cm from the tip. Approximately 0.1 ml of blood was aseptically transferred to the surface of a 5% sheep blood agar plate. Plates were incubated at 37°C in 5% CO<sub>2</sub> for 72 h. A blood culture result was considered positive when colonies revealed  $\alpha$  hemolysis and a mucoid appearance and the gram stain revealed gram-positive diplococci.

#### Statistical Analysis

Survival curves were generated using a commercially available statistical software program (Statistica; StatSoft, Tulsa, OK). Cox's F test was used to compare survival curves. Seven comparisons were made: combined therapy *versus* all the other groups, and intramuscular penicillin therapy *versus* the control group, gas-ventilation group, and partial liquid ventilation group. Bonferroni correction was used for the multiple comparisons. The null hypothesis was rejected for a corrected probability value <0.05.

## Results

#### Pathology/Blood Cultures

At 4 h, the mean lung pathology score of five infected but untreated control rats was 7.2 (range, 6–9). At 12 and 24 h, respectively, the mean scores were 14 (range,

12–16) and 15.8 (range, 13–18). Bacteremia was observed in 25% of rats at 4 h and in 100% of rats at 12 and 24 h. Representative gross and microscopic lung sections are depicted in figure 2.

#### Survival Curves

Survival curves are depicted in figure 3. Between days 4 and 7, mortality was 100% in the control, gas-ventilated, and partial liquid ventilation animals. In the penicillin G benzathine-treated animals, survival was 40% with no death occurring after day 3 ( $P < 0.05$  compared with control and partial liquid ventilation groups). The survival rate for animals treated with partial liquid ventilation and penicillin G benzathine was 80% ( $P < 0.05$  compared with all other groups).

## Discussion

This is the first published study describing the use of PAGE in combination with antibiotic administration for the treatment of pneumonia. The combination of partial liquid ventilation with FC-77 and a systemic antibiotic was superior to treatment with either modality alone.

The beneficial effect of partial liquid ventilation in combination with antibiotic agents on outcome may be due to (1) a direct effect of perfluorocarbon on bacterial growth in the lung; (2) the lavaging of bacteria out of the lung; (3) an antiinflammatory effect of the perfluorocarbon; or (4) improvements in total pulmonary surfactant.<sup>15</sup> We have previously shown, however, that FC-77 does not affect the growth of pneumococci *in vivo* or *in vitro*<sup>16</sup> nor does it appear to lavage bacteria from the lung<sup>16</sup>; therefore, the third and fourth explanations are most likely.

The effects of perfluorocarbons on the inflammatory process are not well understood. Smith *et al.*<sup>14</sup> have shown that production of reactive oxygen species is decreased by lipopolysaccharide-stimulated alveolar macrophages *in vitro* when exposed to perflubron. Nesti *et al.* showed that exposure to perflubron enhanced healing and reduced inflammation of acute full-thickness burns<sup>17</sup> and the gastrointestinal tract in an animal model of ulcerative colitis.<sup>18</sup> Both FC-77 and perflubron are eight-carbon perfluorocarbons; the major difference between the two is a terminal bromide on perflubron. Whether FC-77 also reduces inflammation remains to be shown. If FC-77 possesses antiinflammatory properties, the use of this perfluorocarbon may



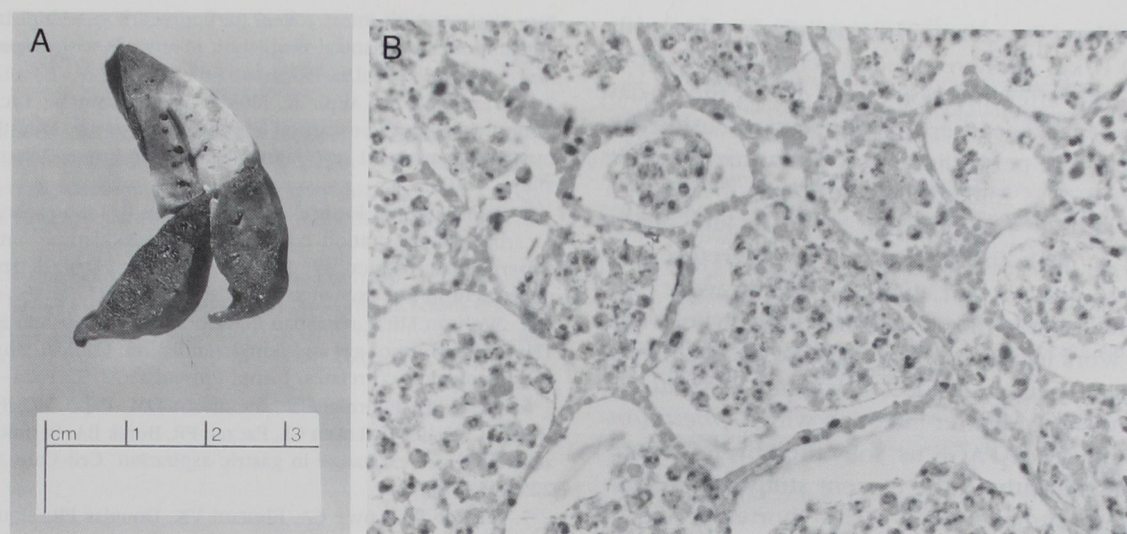


Fig. 2. Representative specimens from an animal with pneumococcal pneumonia. (A) Lung tissue showing gross induration and gray discoloration, upper lobe. (B) Microscopic tissue section showing numerous acute inflammatory cells, mostly polymorphonuclear leukocytes, within alveolar spaces.

provide a wider therapeutic window for systemic antibiotic agents in the treatment of pneumonia.

Pulmonary surfactant is deficient and abnormal in both animal models of pneumonia and clinical studies

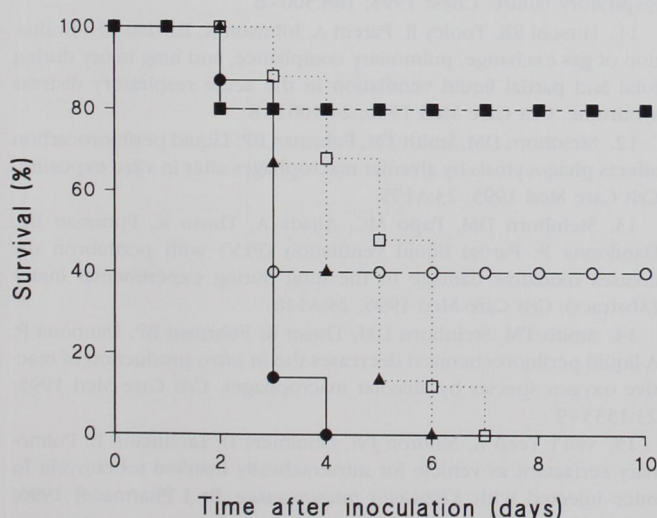


Fig. 3. Survival curve of animals after inoculation (filled circles = no therapy given; open circles = penicillin G benzathine; open squares = partial liquid ventilation; solid triangles = gas ventilation; filled squares = partial liquid ventilation and penicillin G benzathine). There was a significant difference ( $P < 0.05$ ) between the combined treatment group and the other four groups.

of pulmonary infection.<sup>19</sup> Recent data suggest that exposure of type II alveolar cells to perflubron results in increased production of surfactant.<sup>20</sup> FC-77 has a surface tension of 15 dynes/cm<sup>2</sup> at 25°C<sup>21</sup> and may assist as a pulmonary surfactant. This effect may have contributed to the improved outcome in the group receiving combination therapy.

In this study, animals continued to die up to 48 h after initiation of appropriate antibiotic therapy. Penetration of antibiotic agents to the site of infection can be a limitation in the treatment of pneumonia. The delivery of antibiotic agents directly to the lung using perfluorocarbons as a carrier may represent a better therapeutic strategy than either oral or parenteral antibiotic administration. Preliminary data have failed to support this concept, however.<sup>22</sup>

The pneumonia model that was used in this study is characterized by rapid disease progression and a fatal outcome in untreated control rats. All control animals had positive blood cultures and died within 5 days. Histologic examination revealed a consistent, severe pneumonic process. Penicillin G benzathine was chosen as the antibiotic agent because it is highly active against the strain of *S. pneumoniae* used in this study (mean inhibitory concentration [MIC] = 0.02 µg/ml). A single intramuscular dose of 2,500 U/kg in humans causes a rapid rise in serum concentration above the MIC of susceptible strains, with the concentration sus-



tained for at least 2 weeks.<sup>23</sup> The dose used in this study ( $\approx 600,000$  U/kg) likely resulted in serum levels above the MIC shortly after injection to the end of the 10-day study period (package insert). Previous work<sup>8</sup> using this model reveal that penicillin G was uniformly effective when therapy was initiated within 12 h of infection (mortality = 0%), 40% effective at 24 h, and nearly ineffective at 36 h (mortality = 90%); therefore, we chose a time point when antibiotic agents were only partially effective and probably more clinically relevant.

Previous work in our laboratory has shown that partial liquid ventilation alone reduces 5-day mortality rates in animals ventilated 4 h after infection with *S. pneumoniae*.<sup>8</sup> The failure of PAGE as solo therapy to reduce mortality significantly in the current study may reflect the delay in treatment (4 vs. 24 h), shorter period of ventilation (10 vs. 30 min), and the longer period of observation (5 vs. 10 days). Unlike in the previous study,<sup>8</sup> in which animals underwent a tracheotomy, a small-caliber uncuffed endotracheal tube was used in this study. As a result, adequate ventilation (gas or partial liquid) could not be sustained beyond 10 min. It was our intent to provide a period of ventilation sufficiently long to transport the perfluorocarbons into the alveolar space while minimizing upper airway trauma. Although the mechanical ventilation time was decreased, exposure of pulmonary parenchyma to FC-77 continued beyond the period of ventilation as the perfluorocarbons had not specifically drained from the lung. Instead, animals were placed in oxygen chambers and FC-77 dissipated by evaporation. The half-life for FC-77 evaporation in the rat has not been determined. Estimates of perflubron evaporative loss in piglets indicate that approximately 12 h is required for complete evaporation of a functional residual capacity dose.<sup>24</sup>

In this rat model of lethal pneumococcal pneumonia, partial liquid ventilation with FC-77 in combination with penicillin G therapy improved 10-day mortality rates to a greater extent than antibiotic agents or PAGE alone. PAGE is potentially effective in the treatment of the infected lung. Further studies to confirm the safety and efficacy of partial liquid ventilation with perfluorocarbons are warranted.

## References

1. Fuhrman BP, Paczan PR, DeFrancis M: Perfluorocarbon-associated gas exchange. *Crit Care Med* 1991; 19:712-22
2. Tuntuncu AS, Faithfull NS, Lachmann B: Comparison of ventilatory support with intratracheal perfluorocarbon administration and conventional mechanical ventilation in animals with respiratory failure. *Am Rev Respir Dis* 1993; 148:785-92
3. Tuntuncu AS, Akpir K, Mulder P, Erdmann W, Lachmann B: Intratracheal perfluorocarbon administration as an aid in the ventilatory management of respiratory distress syndrome. *ANESTHESIOLOGY* 1993; 79:1083-93
4. Tuntuncu AS, Faithfull NS, Lachmann B: Intratracheal perfluorocarbon administration combined with mechanical ventilation in experimental respiratory distress syndrome: Dose-dependent improvement in gas exchange. *Crit Care Med* 1993; 21:962-9
5. Wolfson MR, Greenspan JS, Deoras KS, Rubenstein SD, Shaffer TH: Comparison of gas and liquid ventilation: Clinical, physiological and histological correlates. *J Appl Physiol* 1992; 72:1024-31
6. Nesti FD, Fuhrman BP, Steinhorn DM, Papo MC, Hernan LJ, Duffy LC, Fisher JE, Leach CL, Paczan PR, Burak BA: Perfluorocarbon-associated gas exchange in gastric aspiration. *Crit Care Med* 1994; 22:1445-52
7. Shaffer TH, Lowe CA, Bhutani VK, Douglas PR: Liquid ventilation: Effects on pulmonary function in distressed meconium-stained lambs. *Pediatr Res* 1984; 18:47-52
8. Dickson EW, Heard SO, Fraire A, Feltquate D, Chu B, Chapman G: Partial liquid ventilation in the treatment of rats with lethal pneumococcal pneumonia (Abstract). *Crit Care Med* 1995; 23:A263
9. Wilcox DT, Glick PL, Karamanoukian HL, Leach C, Morin FC III, Fuhrman BP: Perfluorocarbon-associated gas exchange improves pulmonary mechanics, oxygenation, ventilation and allows nitric oxide delivery in the hypoplastic lung congenital diaphragmatic hernia lamb model. *Crit Care Med* 1995; 23:1858-63
10. Hirschl RB, Tooley R, Parent AC, Johnson K, Bartlett RH: Improvement of gas exchange, pulmonary function, and lung injury with partial liquid ventilation: A study model in a setting of severe respiratory failure. *Chest* 1995; 108:500-8
11. Hirschl RB, Tooley R, Parent A, Johnson K, Bartlett RH: Evaluation of gas exchange, pulmonary compliance, and lung injury during total and partial liquid ventilation in the acute respiratory distress syndrome. *Crit Care Med* 1996; 24:1001-8
12. Steinhorn DM, Smith TM, Fuhrman BP: Liquid perfluorocarbon affects phagocytosis by alveolar macrophages after in vitro exposure. *Crit Care Med* 1995; 23:A195.
13. Steinhorn DM, Papo MC, Aljada A, Thusu K, Fuhrman BP, Dandonna P: Partial liquid ventilation (PLV) with perflubron decreases oxidative damage to the lung during experimental injury (Abstract). *Crit Care Med* 1996; 24:A148.
14. Smith TM, Steinhorn DM, Thusu K, Fuhrman BP, Dandonna P: A liquid perfluorochemical decreases the *in vitro* production of reactive oxygen species by alveolar macrophages. *Crit Care Med* 1995; 23:1533-9
15. van't Veen A, Mouton JW, Gommers D, Lachmann B: Pulmonary surfactant as vehicle for intratracheally instilled tobramycin in mice injected with *Klebsiella pneumoniae*. *Br J Pharmacol* 1996; 119:1145-8
16. Dickson EW, Heard SO, Brueggemann AB, Doern GC: Spontaneous perfluorocarbon ventilation as a treatment for lethal pneumococcal pneumonia in mice (Abstract). *Crit Care Med* 1996; 24:A106
17. Nesti F, Cosio C, Bu JC, Walding D, Smith O: Modulation of epidermal wound healing with perflubron in full thickness burns of the rat (Abstract). *Crit Care Med* 1996; 24:A139



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18. Nesti F, Cosio C, Walding D, Bu JC, Smith O: Modulation of inflammation with perflubron in the murine model of dextran sulfate sodium induced ulcerative colitis (Abstract). *Crit Care Med* 1996; 24:A33
19. Gunther A, Siebert C, Schmidt R, Ziegler S, Grinninger F, Yabut M, Temmesfeld B, Walmrath D, Morr H, Seeger W: Surfactant alterations in severe pneumonia, acute respiratory distress syndrome and cardiogenic lung edema. *Am J Respir Crit Care Med* 1996; 153:176-84
20. Leach CL, Holm BA, Morin FC III, Fuhrman BP, Papo MC, Hernan LJ: Partial liquid ventilation with liquivent increases endogenous surfactant production in premature lamb with respiratory distress syndrome (Abstract). *Pediatr Res* 1995; 39:220A
21. Shaffer TH, Wolfson MR, Clark LC: Liquid ventilation. *Pediatr Pulmonol* 1992; 14:102-9
22. Dickson EW, Heard SO, Goodin T, Brueggemann AB, Doern GV: Use of a penicillin containing perfluorocarbon emulsion in the treatment of rats with lethal pneumococcal pneumonia. *Crit Care Med* 1996; 24:A29
23. Mandell GL, Petri WA Jr: Antimicrobial agents: Penicillins, cephalosporins, and other  $\beta$ -lactam antibiotics, *Goodman and Gilman's Pharmacological Basis of Therapeutics*. Edited by Hardman JG, Limbird LE. New York, McGraw-Hill, 1996, pp 1073-1101
24. Salman NH, Fubman BP, Steinhorn DM, Papo MC, Hernan LJ, Leach CL, Fischer JE: Prolonged studies of perfluorocarbon associated gas exchange and of the resumption of conventional mechanical ventilation. *Crit Care Med* 1995; 23:919-24