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Effect of surfactant replacement on *Pneumocystis carinii* pneumonia in rats

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Abstract. The effect of intratracheal surfactant instillation on pulmonary function in rats with *Pneumocystis carinii* pneumonia (PCP) was investigated. In those animals which developed PCP with severe respiratory failure after administration of cortisone acetate s.c. over 8–12 weeks, pulmonary function was improved by surfactant instillation. PaO_2 values 30 min after surfactant instillation were significantly higher compared to pretreatment values and also compared to PaO_2 values of rats 30 min after receiving saline (482.9 mmHg ± 44.7, 170.7 mmHg ± 39.3 and 67.2 mmHg ± 17.4, respectively). Histological examination showed that alveoli of rats with PCP which received no exogenous surfactant are filled with foamy edema, whereas after exogenous surfactant alveoli are stabilized and well-aerated. These results indicate that exogenous surfactant may help patients with severe PCP to overcome an acute stage of respiratory distress.

Key words: *Pneumocystis carinii* pneumonia – Respiratory failure – Surfactant replacement – Blood gases – Animal model

Infectious diseases are of great concern in intensive care health service in immunocompromized patients; especially infectious diseases of the lung. In these patients *Pneumocystis carinii* is a major cause of pneumonia leading to severe respiratory distress [1, 2]. It has been demonstrated in rats that *Pneumocystis carinii* pneumonia (PCP) decreases the total amount of phospholipids in bronchoalveolar lavage (BAL) fluid whereas phospholipase activity in BAL fluid increases [3, 4]. Furthermore, lung compliance also decreases in PCP [3]. These findings suggest that in the pathophysiology of respiratory failure in PCP the pulmonary surfactant system may be involved.

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It has been repeatedly demonstrated by several authors that intratracheal instillation of exogenous surfactant restores lung function in animals with respiratory failure due to surfactant depletion or intratracheal HCl administration [5, 6]. Also large numbers of neonates with respiratory distress syndrome (RDS) due to lack of surfactant have been successfully treated with exogenous surfactant [7–10].

We designed a study to investigate the effect of surfactant replacement therapy on pulmonary function in rats with PCP. If surfactant replacement would increase gas exchange, then one could conclude that a disturbed surfactant system is one of the factors in the pathophysiology of respiratory failure in PCP.

Materials and methods

Surfactant

The surfactant used in these experiments is a natural surfactant isolated from bovine lungs in basically the same manner as described previously [11]. It consists of approximately 83% phospholipids, 1% hydrophobic proteins (SP-B and SP-C), the remainder being other lipids such as cholesterol, glyceride and free fatty acids. There is no SP-A in this surfactant preparation.

Animal model

This protocol was approved by the Animal Care and Use Committee of the Erasmus University Rotterdam, The Netherlands.

The studies were performed in 28 male Wistar rats (initial body weight: 110–120 g), placed in two groups. The first group ($n = 20$) was treated with cortisone acetate, based on the model described by Frenkel and Chandler [12, 13]. These animals received 12.5 mg cortisone acetate, s.c. 4 times weekly over 8–12 weeks and 10 µg/ml doxycycline in drinking water to reduce a possible bacterial superinfection. The second group ($n = 8$) received no treatment and served as controls. All animals were housed in standard plastic cages and received standard food ad libitum and were weighed at weekly intervals.

After 8–12 weeks the cortisone acetate treated animals developed respiratory failure as judged by clinical symptoms (e.g. tachypnoea, cyanosis and fast decrease in body weight). Eleven animals died during

the night hours on various days before treatment with exogenous surfactant could be started. Nine rats which suffered clinically from respiratory failure were anesthetized with pentobarbital sodium (60 mg/kg, i.p.), tracheotomized and paralyzed with pancuronium bromide (0.5 mg/kg, i.m.). A catheter was inserted into the carotid artery for blood sampling. Animals were ventilated pressure-controlled with a Servo Ventilator 900 C (Siemens-Elema, Solna, Sweden) at the following settings: $FiO_2 = 1.0$, ventilation frequency = 30/min, peak airway pressure = 22 cm H_2O, PEEP = 2 cm H_2O and I:E = 1:2.

After a ±20 min stabilization period, 6 animals received bovine surfactant (200 mg dry weight/kg body weight), suspended in 0.7 ml saline, intratracheally, whereas 3 received the same amount of saline. Blood samples were taken from the carotid artery immediately before surfactant instillation and at 30 and 60 min post treatment. Blood gases were measured with the ABL 330 Acid-Base Laboratory (Radiometer, Copenhagen, Denmark).

Histological examination

At the end of the experiments the animals were sacrificed with an overdose of intra-arterially administered pentobarbital. The lungs were removed, the left lobe was minced and a cytospin preparation was made. This preparation was stained with a modified Gomori stain [14], which allows visualization of the cysts (not the intra-cystic structures) and Giemsa stain, and examined to confirm presence of PCP. The right lobes were fixed in 10% formalin for light microscopy examination, dehydrated, embedded in paraffin and 6 µm sections of the right middle lobe were stained with hematoxylin and eosin, Grocott-Gomori methenamine silver nitrate and periodic acid-Schiff stain.

Statistical analysis

Statistical evaluation of data was performed using the Wilcoxon signed rank test for paired observations or the Mann-Whitney-U test (two-tailed). All data are expressed as mean ± SEM. Statistical significance was accepted at $p < 0.05$ (two-tailed).

Results

Blood gases

During an 8–12 weeks period the animals developed respiratory insufficiency which could be reversed by surfactant instillation. PaO_2 values at 30 and 60 min after surfactant instillation are significantly higher compared to pre-treatment values. There was no significant difference between PaO_2 values after surfactant treatment and PaO_2 values of healthy control animals, though the latter were somewhat higher. Intratracheal instillation of only saline, however, led to a decrease of PaO_2 compared to pre-treatment PaO_2 values. The difference at 30 and 60 min between rats receiving surfactant and rats receiving saline is significant. Pre-treatment PaO_2 values of both groups do not differ significantly (Fig. 1). Figure 2 shows one example of a dramatic increase of PaO_2 of a rat with PCP treated with exogenous surfactant. This rat had the lowest PaO_2 of the whole group.

Histological findings

Histological examination of lungs of rats with PCP which received only saline and no exogenous surfactant showed alveoli filled with a characteristic foamy edema (Fig. 3). Surfactant treatment resulted in stabilized and aerated alveoli, thus allowing the emphysematous structure of the lungs to become apparent (Fig. 4).

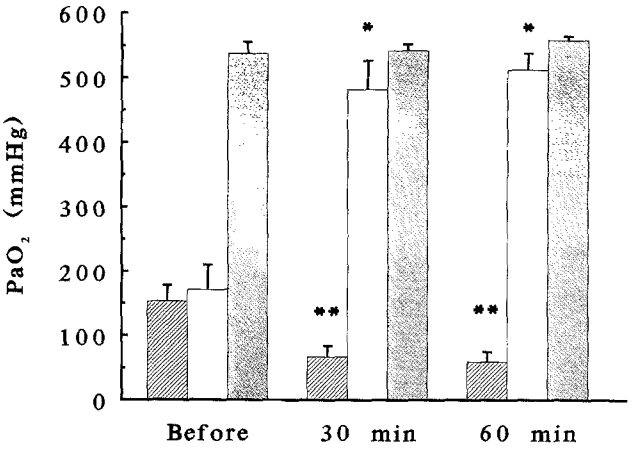

Fig. 1. PaO_2 values of the different groups before treatment ("Before") and 30 min and 60 min after treatment (respectively: "30 min" and "60 min"). *Grey bar* = healthy controls; *striped bar* = saline group; *open bar* = exogenous surfactant group. (* = significantly different from pre-treatment; ** = significantly different from surfactant treated rats ($p < 0.05$))

In all 9 lung homogenates a large amount of cysts was present which confirmed the presence of PCP in all animals.

Discussion

The treatment of choice in patients suffering from respiratory failure due to PCP is administration of antimicrobial drugs combined with ventilatory support. The aim of the present study was to investigate the effect of an exogenous surfactant preparation on gas exchange under unchanged ventilator settings in an animal model of PCP.

The primary functions of surfactant are stabilization of alveoli of different size at end expiration and reduction of work of breathing by reducing surface tension at the air-liquid interphase on the alveolar walls [15]. In this study two hypotheses were proven valid: *hypothesis 1)* if surfactant is necessary to keep retractive forces as low as

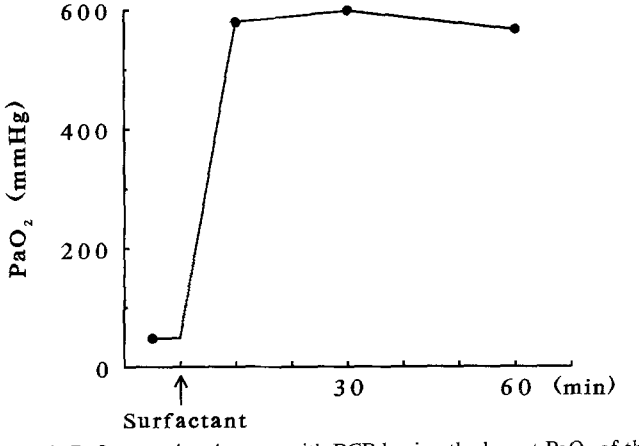

Fig. 2. PaO_2 over time in a rat with PCP having the lowest PaO_2 of the whole group before surfactant treatment: in this animal PaO_2 was also measured 10 min after surfactant instillation. The *arrow* indicates the time of surfactant instillation

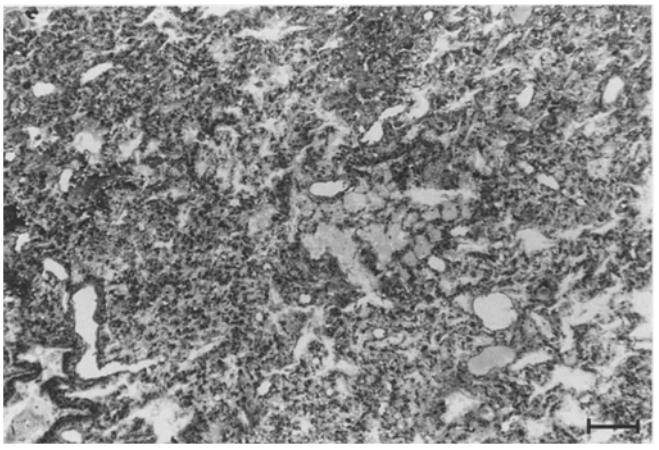

Fig. 3. Typical histological findings in a rat with PCP which received saline instead of exogenous surfactant. Hematoxylin and eosin stain; the scale-line equals 100 μm

possible and to ensure optimal gas exchange, any disturbance in the surfactant system will result in abnormalities in lung distensibility and gas exchange; *hypothesis 2)* if the first hypothesis is correct, surfactant replacement will restore lung distensibility and gas exchange to normal. In other words, if surfactant replacement improves decreased lung compliance and impaired gas exchange in lung diseases of different etiologies, then the surfactant system is also involved in the pathogenesis of these lung diseases [16].

The results of this study demonstrate that surfactant instillation in rats with respiratory failure due to *Pneumocystis carinii* infection leads to restoration of pulmonary gas exchange, which could be demonstrated by improvement of blood gases and by histological examination. These results underline our hypothesis that the pulmonary surfactant system is at least partly responsible for the respiratory failure caused by PCP. The mechanism can be explained as follows: as a result of damage to the alveolar-capillary membrane caused by infection with *Pneumocystis carinii*, large volumes of edema fluid accumulate into the alveolar space. This edema contains large amounts of plasma proteins and specific enzymes capable of disrupting the surfactant system [3, 4, 17–19]. As a result of this functional surfactant deficit, surface tension at the airliquid interphase on the alveolar wall increases, leading to increased retractive force across the alveolar-capillary membrane, with subsequent formation of atelectatic areas, functionally expressed as a decrease in functional residual capacity (FRC). This leads to a mismatch of the ventilation-perfusion ratio resulting in hypoxemia. Also, due to increase of surface tension on the alveolar walls, suction force across the alveolar-capillary membrane increases, facilitating a further outpouring of edema [20]. The mechanism by which surfactant replacement therapy leads to improvement of gas exchange can be explained by reversing these mechanisms. The fact that gas exchange improves only after administration of surfactant and not after saline indicates that the positive effect observed is caused by surfactant and not by the fluid itself.

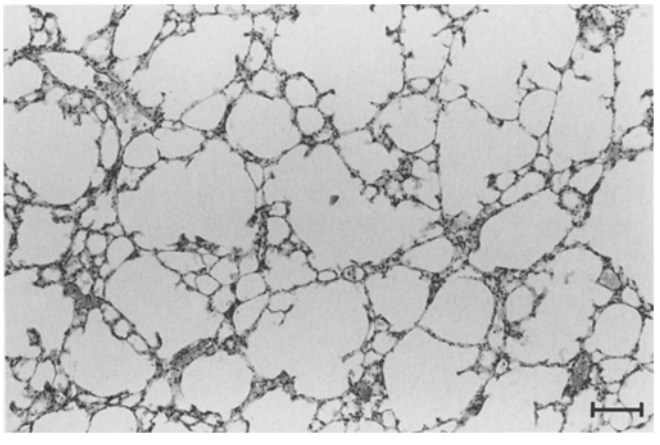

Fig. 4. Typical histological findings in rats with PCP after treatment with surfactant; note the emphysematous structure. Hematoxylin and eosin stain; the scale-line equals 100 μm

In all animals treated with exogenous surfactant it was also found that many alveolar septa were damaged, leading to an emphysematous lung structure. Similar observations were reported recently in patients with PCP [21]. These findings suggest that in PCP lungs proteolytic enzymes may be over-represented. These proteolytic enzymes are perhaps excreted by activated leukocytes attracted to the site of inflammation. Also in the past it has been found that type I pneumocytes undergo degenerative changes in rats infected with *Pneumocystis carinii.* This leads to damage to the ultrastructure of the alveolar wall, thus leading to increased permeability of the alveolar-capillary membrane [22]. Perhaps both mechanisms play a role in the damage of the alveolar septa. It could be speculated that proteinase inhibitors, given to the patient after manifestation of pneumocystis carinii infection, could prevent (further) morphological damage to the pulmonary structure.

It is established that immunosuppressed rats, as is the case in humans, can develop *Pneumocystis carinii* infection of the lungs, leading to respiratory distress. The current treatment of choice is parenteral administration of trimethoprim-sulfamethoxazole or pentamidine-isothionate. Both drugs have about the same rate of success of around 75% (percentages vary depending on the study) but high incidences of systemic adverse reactions are reported (for review see [23]). To prevent these adverse reactions it is necessary to lower the systemic concentration, which led to studies on aerosolized pentamidine. Results from these aerosol studies have shown that it is possible to get high concentrations in the lungs with low systemic concentrations [24, 25]. For the future one could speculate to use surfactant as a carrier-substance for antimicrobial drugs, this way achieving high intra-alveolar drug concentration and low systemic concentration.

In conclusion, it has been demonstrated that surfactant instillation can improve gas exchange in rats suffering from respiratory failure due to PCP. These results could be important in future for treatment regimens of patients suffering from acute respiratory failure due to PCP.

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References

1. CDC Update (1984) Acquired immuno-deficiency syndrome (AIDS)-United States. MMWR 32:688–691
2. Walzer PD, Perl DP, Krogstad DJ, Rawson PG, Schultz MG (1974) *Pneumocystis carinii* pneumonia in the United States: epidemiologic, diagnostic, and clinical features. Ann Intern Med 80:83–93
3. Sheehan PM, Stokes DC, Yeh Y, Hughes WT (1986) Surfactant phospholipids and lavage phospholipase A_2 in experimental *pneumocystis carinii* pneumonia. Am Rev Respir Dis 134:526–531
4. Kernbaum, S. Masliah J, Alcindor LG, Bouton C, Christol D (1983) Phospholipase activities of bronchoalveolar lavage fluid in rat pneomocystis carinii pneumonia. Br J Exp Pathol 64:75–80
5. Berggren P, Lachmann B, Curstedt T, Grossmann, G, Robertson B (1986) Gas exchange and lung morphology after surfactant replacement in experimental adult respiratory distress syndrome induced by repeated lung lavage. Acta Anaesthesiol Scand 30:321–328
6. Kobayashi T, Ganzuka M, Taniguchi J, Nitta K, Murakami S (1990) Lung lavage and surfactant replacement for hydrochloric acid aspiration in rabbits. Acta Anaesthesiol Scand 34:216–221
7. Fujiwara, T, Chida, S, Watabe Y, Maeta H, Morita T, Abe T (1980) Artificial surfactant therapy in hyaline-membrane disease. Lancet I:55–59
8. Hallman M, Merritt TA, Jarvenpaa A-L, Boynton B, Mannino F, Gluck L, Moore T, Edwards D (1985) Exogenous human surfactant for treatment of severe respiratory distress syndrome: a randomized clinical trial. J Pediatr 106:963–969
9. Collaborative European Multicenter Study Group (1988) Surfactant replacement therapy for severe neonatal respiratory distress syndrome: an international randomized clinical trial. Pediatrics 82:683–691
10. Ten Centre Study Group (1987) Ten centre trial of artificial surfactant (artificial lung expanding compound) in very premature babies. Br Med J 294:991–996
11. Metcalfe IL, Enhorning G, Possmayer F (1980) Pulmonary surfactant-associated proteins: their role in the expression of surface activity. J Appl Physiol 49:34–41
12. Frenkel JK, Good JT, Schultz JA (1966) Latent pneumocystis infection of rats, relapse, and chemotherapy. Lab Invest 15:1559–1577
13. Chandler FW, Frenkel JK, Campbell WG (1979) Animal model of human disease: *pneumocystis* pneumonia. Am J Pathol 95:571–574
14. Musto L, Flanigan M, Elbadawi A (1982) Ten-minute silver stain for *pneumocystis carinii* and fungi in tissue sections. Arch Pathol Lab Med 106:292–294
15. Clements JA (1977) Functions of the alveolar lining. Am Rev Respir Dis 115:67–71
16. Lachmann B (1988) Surfactant replacement in acute respiratory failure: animal studies and first clinical trials. In: Lachmann B (ed) Surfactant replacement therapy in neonatal and adult respiratory distress syndrome. Springer, Berlin, pp 212–223
17. Ikegami M, Jobe A, Jacobs H, Lam R (1984) A protein from airways of premature lambs that inhibits surfactant function. J Appl Physiol 57:1134–1142
18. Holm BA, Enhorning G, Notter RH (1988) A biophysical mechanism by which plasma proteins inhibit lung surfactant activity. Chem Phys Lipids 49:49–55
19. Seeger W, Stöhr G, Wolf HRD, Neuhof H (1985) Alteration of surfactant function due to protein leakage: special interaction with fibrin monomer. J Appl Physiol 58:326–338
20. Guyton AC, Moffat DS, Adair TH (1984) Role of alveolar surface tension in transepithelial movement of fluid. In: Robertson B, Van Golde LMG, Batenburg JJ (eds) Pulmonary surfactant. Elsevier, Amsterdam, pp 171–185
21. Beers MF, Sohn M, Swartz M (1990) Histological evidence of peripheral tissue destruction in AIDS patients with *pneumocystis carinii* pneumonia complicated by recurrent spontaneous pneumothorax. Am Rev Respir Dis 141 [Suppl]:A273
22. Yoneda K, Walzer PD (1981) Mechanism of pulmonary alveolar injury in experimental *pneumocystis carinii* pneumonia in the rat. Br J Exp Pathol 62:339–346
23. Levine SJ, White DA (1988) *Pneumocystis carinii.* Clin Chest Med 9:395–423
24. Montgomery AB, Debs RJ, Luce JM, Corkery KJ, Turner J, Brunette EN, Lin ET, Hopewell PC (1987) Aerosolized pentamidine as sole therapy for *pneumocystis carinii* pneumonia in patients with acquired immunodeficiency syndrome. Lancet II:480–483
25. Debs RJ, Blumenfeld W, Brunette EN, Straubinger RM, Montgomery AB, Lin E, Agabian N, Papahadjopoulos D (1987) Successful treatment with aerosolized pentamidine of *pneumocystis carinii* pneumonia in rats. Antimicrob Agents Chemother 31:37–41

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