VIA MEDICA
SHORT COMMUNICATION
Vol. 62, No. 4, pp. 377–379

ISSN 0015–5659
www.fm.viamedica.pl

Localisation of exogenous surfactants in cell membranes in the air-blood barrier: rat model

Andrzej Marszałek[1], Wiesława Biczysko[1], Marcin Wąsowicz[2], Paweł Surowiak[3, 4], Maciej Zabel[3], Ewa Florek[5]

[1]*Department of Clinical Pathomorphology, University of Medical Sciences, Poznań, Poland*
[2]*Department of Anesthesiology and Intensive Care, Collegium Medicum, Jagiellonian University, Kraków, Poland*
[3]*Department of Histology and Embryology, Medical Academy, Wrocław, Poland*
[4]*Charite Medical University, Berlin, Germany*
[5]*Department of Toxicology, University of Medical Sciences, Poznań, Poland*

[Received 17 September 2003; Accepted 22 October 2003]

The use of exogenous surfactants has been introduced into the therapy of patients of different ages. Much better results have been obtained in the treatment of respiratory distress syndrome with surfactants enriched with surfactant proteins. In the following study we used protein-containing surfactants (survanta and curosurf). The aim of the following study was to determine the localisation of artificial surfactants in the lung tissue. Using the Immunogold Technique, biotinylated surfactant proteins were traced in the air-blood barriers. In all lungs the exogenous surfactant was present only in some alveoli. In these parts small areas of atelectasis as well as oedema and transudate accumulation were seen. These changes were less severe after biotinylated curosurf treatment. In electron microscope studies we found surfactant elements in the air-blood barrier and other structures of the alveolar septa. Immunogold studies confirm the presence of biotynylated surfactant in the elements of the air-blood barrier.

key words: exogenous surfactant, lung, electron microscopy, immunogold

INTRODUCTION

The first artificial surfactants were used in the therapy of respiratory distress syndrome in neonates. Now, they are also used in the prevention and treatment of ARDS [2, 8]. Most data on the efficiency of surfactants used in stabilisation at the air-fluid phase are from *in vitro* studies [4]. An improvement in lung aeration after surfactant instillation is well known. However, side effects, such as pulmonary haemorrhages, brain haemorrhages, increased lung fibrosis and even lung cavity formation with accompanied infections, were also described [7]. The long-term effects of surfactant therapy are still incomplete and the results ambiguous [4, 7]. The question as to how the exogenous surfactants can have any influence on the development of alveoli or the remodelling of mature ones remains an open one. Controlled *in vivo* experiment with observation of the stages of injury, regeneration and parenchymal rebuilding of the lung has been incomplete. The aim of this study was the tracing of exogenous surfactants within the air-blood barrier structures in 24-hour observations.

MATERIAL AND METHODS

We used young adult pathogen-free rats of the Wistar strain (body weight 240–250 g). After anaesthesia (narkotan) the animals were intubated and a dose of surfactant (100 or 150 mg of lipids per kg

Address for correspondence: Andrzej Marszałek, Department of Clinical Pathomorphology, University of Medical Sciences, ul. Przybyszewskiego 49, 60–355 Poznań, Poland, e-mail: gensui@wp.pl

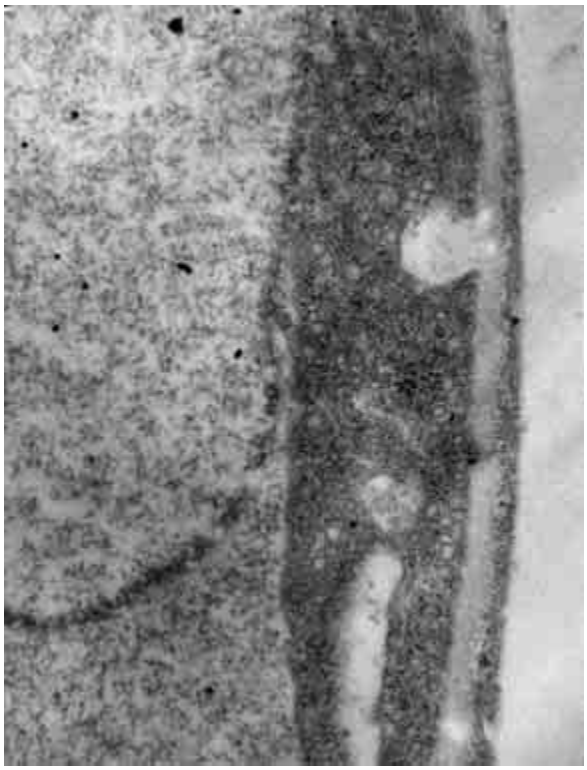

Figure 1. Part of the thin air-blood barrier. Anti-biotin-gold conjugated particles are visible the epithelial cell membrane, in endothelial cytoplasm, and within capillary lumen. Immunogold. Primary magnification 12,000 ×.

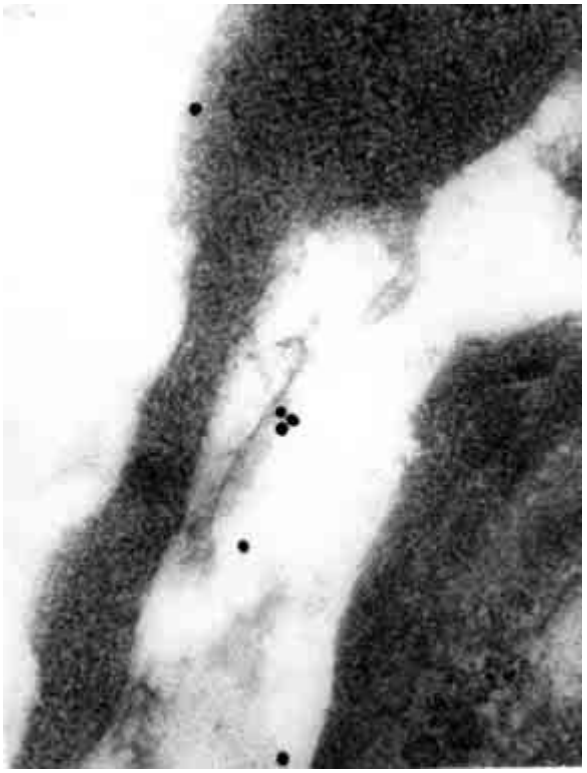

Figure 2. Part of the alveolar septum. Anti-biotin-gold conjugated particles are found on the cell surface and beneath the epithelium on the parts of the surfactant. Immunogold. Primary magnification 50,000 ×.

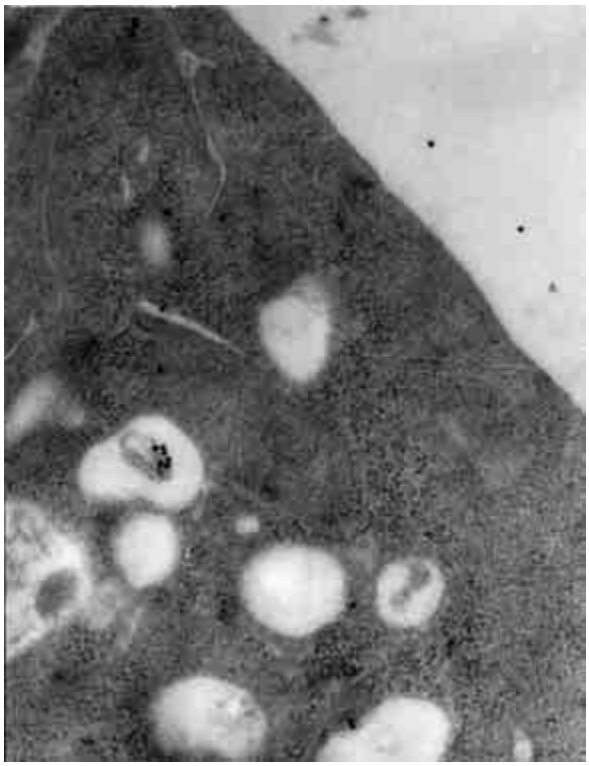

Figure 3. Anti-biotin-gold conjugated particles are found within the vacuole, on cell surface, and beneath the epithelium on the parts of surfactant. Immunogold. Primary magnification 20,000 ×.

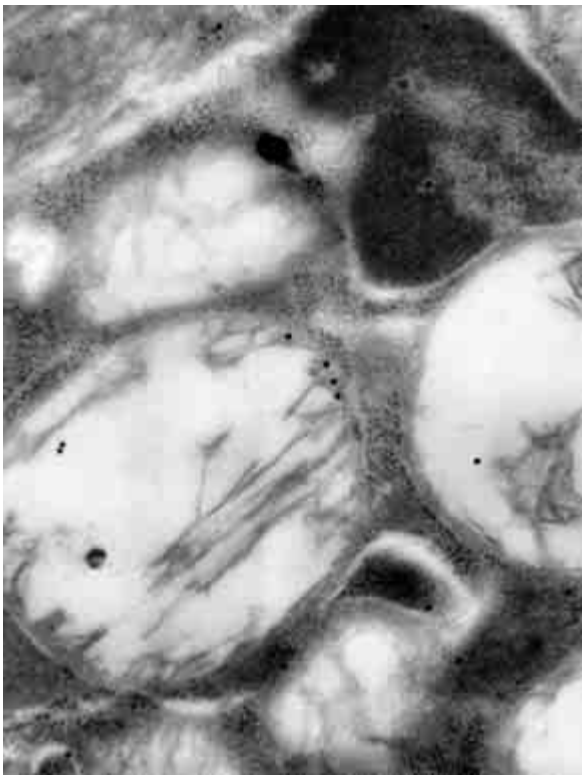

Figure 4. Anti-biotin-gold conjugated particles are found within the lamellar body. Immunogold. Primary magnification 20,000 ×.

of body weight; survanta and curosurf or biotinylated curosurf respectively) was introduced directly to the trachea. Controls were given an equivalent volume of 0.9% NaCl. After 1, 3, 6, and 24 hours the animals were sacrificed and the lungs removed and processed in a routine procedure, the left lung into paraffin blocks and the right into epon 812 blocks. There were not less then 7 and 4 animals respectively in each of the studied groups and controls. For the etching of resin for immuno-electron microscopy we used a method with 3% sodium metaperiodate described earlier [6] and then one-step immuno-reaction was performed (goat anti-biotin 20 nm gold conjugated; ICN Biomedicals, Inc.).

RESULTS AND DISCUSSION

The exogenous surfactants introduced intratrachealy were distributed in the lungs only in a disperse pattern. We were unable to record a uniform spread of surfactants within the lung. In those parts where the surfactant entered the alveoli, areas of athelectasis as well as oedema and transudate accumulation were observed. Erythrorrhagias were even observed in some cases (24 hours, survanta and non-biotinylated curosurf). Such changes were not observed in controls. Surfactant structures were observed within the structures of the thin air-blood barriers, alveolar septa and within the capillary lumen (Fig. 1, 2). In routine transmission electron microscopy inclusions of surfactant particles into cell membrane systems was observed. This was confirmed by Immunogold. Some of the particles of biotinylated curosurf were found within lamellar bodies of type II pneumocytes (Fig. 3, 4).

Respiratory distress syndrome in neonates and adults has a high mortality rate [3, 9]. Although clinical data describe the benefits of an acute phase of the treatment, observations over a long period are still incomplete [4]. Surfactants decrease the surface tension (enabling gas exchange) and enhance the transport of oedematous fluid into alveoli [1]. But the excess of surfactants could lead to some damage of the cell membranes [10] and side effects including haemorrhages [3]. Curosurf, containing neutral lipids and specific proteins SP-B and SP-C, is the surfactant preparation now used most widely in Europe. Experimental studies revealed that curosurf forms a monolayer on the water surface very quickly ("adsorption with click") that is due to the high lipid concentration in a small volume. The ability to form a monolayer is the key to its proper function. But the vast majority of publications on this process are based only on *in vitro* models and the results are confusing [4, 5]. This study confirms the recycling of exogenous surfactants into lamellar bodies. Moreover, biotinylated curosurf particles were found in the monolayer of surfactant as well as in the cell membrane systems. The clinical and experimental studies conducted so far [9] have revealed that in some cases lung fibrosis follows the introduction of exogenous surfactants. The recycling of exogenous surfactants may lead to increased formation of lamellar bodies and their release and then to increased macrophage stimulation. According to our previous data, surfactant recirculation and an increase in the number of macrophages laden with surfactant last for about 3 weeks [10]. The concept that the surfactant-laden macrophages contain the exogenous surfactant preparations for so long requires further study.

ACKNOWLEDGEMENTS

This research was supported in part by grant no. 3 P05A 057 25 from KBN.

REFERENCES

1. Biczysko W, Wąsowicz M, Marszałek A, Florek E (1995) The entrance of surface active material to pulmonary capillaries in the lungs of newborn rats. Folia Histochem Cytobiol, 33: 25–31.
2. Bjerklund LJ, Ingimarsson J, Curstedt T, Lersson A, Robertson B, Werner O (2001) Lung recruitment at birth does not improve lung function in immature lambs receiving surfactant. Acta Anaesthesiol Scand, 45: 986–993.
3. British Columbia Reproductive Care Program (1993).
4. Committee on Fetus and Newborn, American Academy of Pediatrics (1999) Surfactant replacement therapy for RDS. Pediatrics 103: 684–685.
5. Ding J, Takamoto DY, vonNahmen A, Lipp MM, Lee KY, Waring A, Zasadzinski JA (2001) Effects of lung surfaktant proteins, SP-B, SP-C and palmitic amic on monolayer stability. Biophys J, 80: 2262–2272.
6. Matsunaga M, Lemura I, Tamura M, Niemoto SI (2002) Role of specialized microvilli and the fertilization envelope in spatial positioning of blastomeres in early development of embryos of the starfish *Astropecten scoparius*. Biol Bull, 202: 213–222.
7. Takamoto DY, Lipp MM, vonNahmen A, Lee KY, Waring A, Zasadzinski JA (2001) interaction of lung surfaktant proteins with anionic phospholipids. Biophys J, 81: 153–169.
8. Tobin MJ (2001) Pediatrics, surfactant and cystic fibrosis in AJRCCM 2000. Am J Respir Crit Care Med, 164: 1581–1594.
9. Wąsowicz M, Biczysko W, Darwiła R, Marszałek A, Andres J, Florek E (2002) Surfaktant egzogenny a uszkodzenie bariery krew-powietrze. Studium eksperymentalne. Anest Inten Ter, 34: 76–80.
10. Żurawski J, Wąsowicz M, Marszałek A, Florek E, Biczysko W (2002) Effect of exogenous pulmonary surfactant preparations on the structure of pulmonary alveoli in newborn rats. Folia Histochem Cytobiol, 40: 285–295.