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Influence of corticosteroids on formation and stability of thin liquid films from pulmonary therapeutic preparations

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Drugs containing corticosteroids are used for treatment of lung inflammations. Due to corticosteroid's amphiphilic nature, they are surface active molecules which are able to modify surface properties of pulmonary surfactant. In the present study natural and synthetic pulmonary therapeutic preparations (PTP) are used as a model of the (lung) pulmonary surfactant. Effect of corticosteroids on surface activity of PTP is evaluated by the black foam film method and probability for formation of black foam films stabilized with PTP in the presence of corticosteroids is assessed. Stable black foam films are formed at higher concentrations. Disjoining pressure isotherms indicate an increase of film thickness in mixtures from PTP and corticosteroids. The results suggest that in the presence of corticosteroids, a mixed adsorption layer of the pulmonary surfactant is formed. Effect observed is stronger in synthetic PTP.

Key words: *thin liquid foam films, pulmonary surfactant, corticosteroids*

INTRODUCTION

Pulmonary surfactant (PS) is a complex lipid-protein mixture [1]. PS forms a variety of thin films at the alveolar surface and prevent alveoli collapse during the process of breathing. The PS consists of lipids and specific proteins (SP-A, SP-B, SP-C, and SP-D). Polar phospholipids (mainly dipalmitoylphosphatidylcholine (DPPC), phosphatidylglycerol, represent about 70% of the pulmonary surfactant. Respiratory distress syndrome (RDS) is a significant cause of morbidity and mortality in preterm infants. RDS is caused by a deficiency, dysfunction, or inactivation of pulmonary surfactant. Pulmonary therapeutic preparations (PTP) are drugs that are administered by instillation into the trachea and they are used to treat RDS in neonates and adults.

Corticosteroids are anti-inflammatory drugs that can be used in asthma to reduce airway hyper-responsiveness and to decrease bronchial edema and mucus secretion [2]. In clinical practice, PTP and corticosteroids are often used together. Their simultaneous use shows larger effect in comparison with their separate application. Administration of PTP and Budesonide enhance lung functions and alleviate inflammation more effectively than Budesonide-only and surfactant-only treatment, as reported in Mikolka et al. [3]. PTP are used as carriers of medical drugs to lung epithelium [4, 2]. So far little is known about the interaction between these medicines and complexes of pulmonary surfactant [4]. Therefore, the impact of corticosteroids on pulmonary surfactant (PS) is of great interest.

Recently, the advantageous simultaneous application of corticosteroids and PTP has been reported [5]. PTPs are used as carriers of corticosteroids to deliver them to the lung epithelium [4, 5]. Identified advantages refer to avoiding first-pass metabolism and eliminating potential side effects caused by high systemic dosage. This makes pulmonary drug delivery an ideal method for treating respiratory diseases, such as asthma, chronic pulmonary infection, emphysema, cystic fibrosis, pulmonary hypertension, and lung cancer [5]

Wang et al. [5] have investigated biophysical interaction between natural PTP Infasurf and two corticosteroids: Budesonide and Beclomethasone dipropionate. Their interaction is assessed by the surface activity measurements by the Langmuir balance and lateral film structure studied by atomic force microscopy suggesting an optimal concentration range of corticosteroids for pulmonary delivery. Infasurf may carry less than 1% Budesonide and up to 10% Beclomethasone without significantly compromising its surface activity [5]. The use of corticosteroids is benefital in the case of asthma and croup, but their effect could be controversial in other diseases [6]. Investigation of clinically used PTP as carriers of corticosteroids and their influence on PTP properties are important from a practical point of view. The standard protocols of corticosteroid applications should be followed, and physicians should be alerted to the potential hazards of their prolonged use, as reported in [6]. Curosurf, a natural porcine surfactant, combined with

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Beclomethasone at 800 μg/kg, is effective in reducing the oxidative lung stress and improving the respiratory function in preterm lambs with RDS, as reported in Dani et al. [7].

In our study we investigate the effect of two corticosteroids, Budesonide and Beclomethasone, on Curosurf, a natural-origin PTP, and on a synthetic PTP containing two phospholipids and two peptides analogues of human surfactant proteins B and C, using the thin liquid foam film methods.

EXPERIMENTAL

Natural and synthetic PTP are used as a model of pulmonary surfactant. As a natural PTP we used Curosurf, provided by Chiesi Pharmaceuticals, Italy. Curosurf preparation is obtained from porcine lung mince and consists of approximately 98% phospholipids and 2% hydrophobic surfactant proteins (SP-C and SP-B). It is provided as an aqueous suspension with concentration of 80 mg/ml in physiological electrolyte concentration (0.15 M NaCl). For the purposes of the study, a stock solution with concentration 800 μg/ml at the same electrolyte concentration was prepared from the ready-made suspension and further used to prepare the working solution with the respective concentration.

Synthetic PTP consists of 98.3% (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) DPPC and (palmitoyl-oleoyl-sn-glycero-3-phosphoglycerol) POPG in a ratio (DPPC:POPG=1:1) and two synthetic peptide analogues of SP-C (1.5%) and SP-B (0.2%) [8].

Corticosteroid stock solutions of 1.5 mg/ml dimethyl sulfoxide (DMSO) were prepared.

Studies of microscopic foam films were carried out with modernized microinterferometric method of Scheludko and Exerowa. To study the probability of black foam film formation, we used a cell with a cylindrical holder. In this cell, the film was formed at constant capillary pressure, when two surfaces of a biconcave drop approach one another. The measurements of the disjoining pressure isotherms were carried out with Exerowa-Scheludko cell with porous plate [9-11], enabling application of variable pressure. The foam film was formed in the orifice of the porous plate when pressure was applied.

Using the thin liquid foam film method we carried out investigation of probability (W) of black foam film formation on surfactant concentration (C). In general, probability is determined experimentally as the ratio of the number of films in which black films are observed ΔN to the total number of studied films N, and can be expressed as percentage or parts of a unit. The two most important characteristics obtained from a W(C)-curve are critical concentration (C_{cr}) and threshold concentration (C_t). C_{cr} is defined as the highest concentration at which all films are ruptured without black film formation (W=0). C_t is the lowest concentration above which all films become black (W=1).

Film stability was determined by observing films at two different waiting times (30 min and 60 min), the waiting time being defined as the time for which the solution is left as a drop in the cylinder holder of measuring cell before film formation.

RESULTS AND DISCUSSION

The films from natural and synthetic PTP and their mixtures with Beclomethasone were studied. Film destabilization was not observed for the synthetic and natural PTP at both corticosteroid concentrations studied. At lower corticosteroid concentration (3 μg/ml Beclomethasone), there was no significant difference between C_{cr} for mixtures and for the pure natural preparation. C_t for mixture shifted to lower concentrations of PTP (Fig. 1)

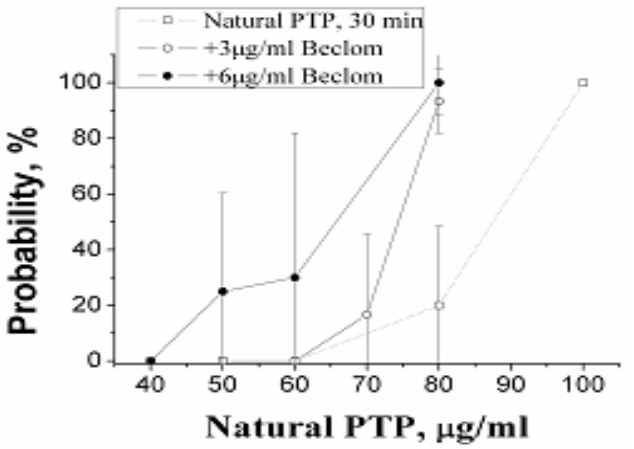

Fig. 1. Probability of formation of black foam films stabilized with natural PTP and mixture of natural PTP with added 3 μg/ml Beclomethasone and 6 μg/ml Beclomethasone at 30 min waiting time.

At higher concentrations of Beclomethasone (6 μg/ml), W(C_S) curves for natural PTP (Fig. 1) shifted towards lower concentrations (i.e. the films are stabilized). For synthetic PTP the same dependency was observed but the scattering of results was larger (data not shown). This scattering might be due to uneven saturation of film interfaces and disruption of aggregates coming from the solution bulk when they last approach the surfaces.

The probability of black foam films formation for pure natural and synthetic PTP and for their mixtures with 3 μg/ml Budesonide and 6 μg/ml Budesonide at 30 min and 60 min waiting time,

were also investigated. At 3 μg/ml Budesonide at both 30 min and 60 min waiting times for synthetic PTP, the W(C_S)-curves (data not shown) shifted to higher surfactant concentrations (i.e. destabilization of films was observed). At 6 μg/ml Budesonide, W(C_S) curves (data not shown) shifted to lower surfactant concentrations (i.e. stabilization of films was observed). For synthetic PTP, within this Budesonide concentration interval, alteration in Budesonide concentration effect on the stability of films was observed.

C_{cr} and C_t for natural PTP practically coincided with these for pure PTP and its mixtures with 3 μg/ml Budesonide at both studied waiting times 30 min and 60 min (data not shown) (i.e. change in film stability after addition of corticosteroids was not observed).

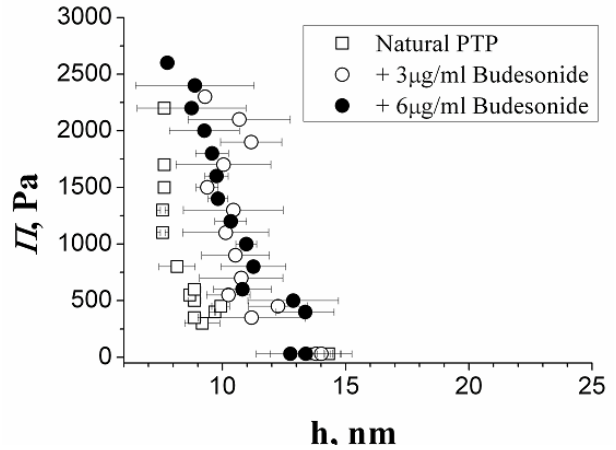

Fig. 2. Disjoining pressure isotherm of natural PTP and its mixture with 3 μg/ml Budesonide (open symbols) and with 6 μg/ml Budesonide (filled symbols).

Π(h)-disjoining pressure isotherms of pure natural and synthetic PTP and of their mixtures with corticosteroids were measured. Fig. 2 depicts *Π*(h)-isotherms for natural PTP in the presence of 3 μg/ml and 6 μg/ml Budesonide and Fig. 3 – at 3 μg/ml and 6 μg/ml Beclomethasone. As it can be seen in Fig. 2, the isotherms of natural PTP Curosurf with added 3 μg/ml and with 6 μg/ml Budesonide practically coincide. They shifted to higher film thicknesses compared to isotherm of pure natural PTP Curosurf.

For synthetic PTP we measured isotherms in the presence of 6 μg/ml Budesonide (Fig. 4) and 6 μg/ml Beclomethasone (Fig. 5). Corticosteroid concentrations were selected to be the same as concentrations at which W(C_S) curves were obtained.

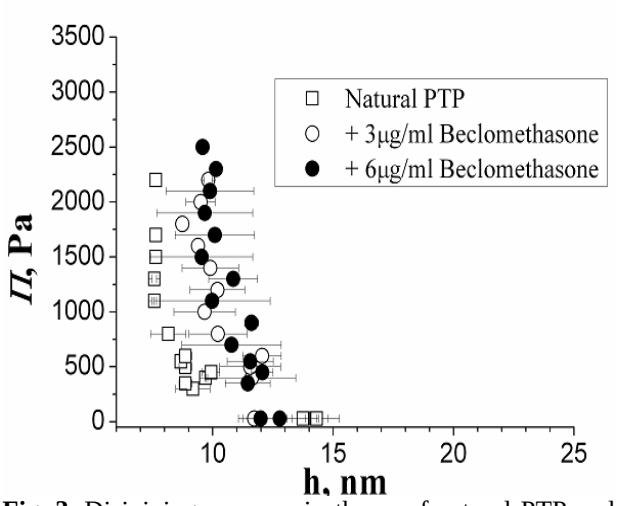

Fig. 3. Disjoining pressure isotherm of natural PTP and its mixture with 3 μg/ml Beclomethasone (open symbols) and with 6 μg/ml Beclomethasone (filled symbols).

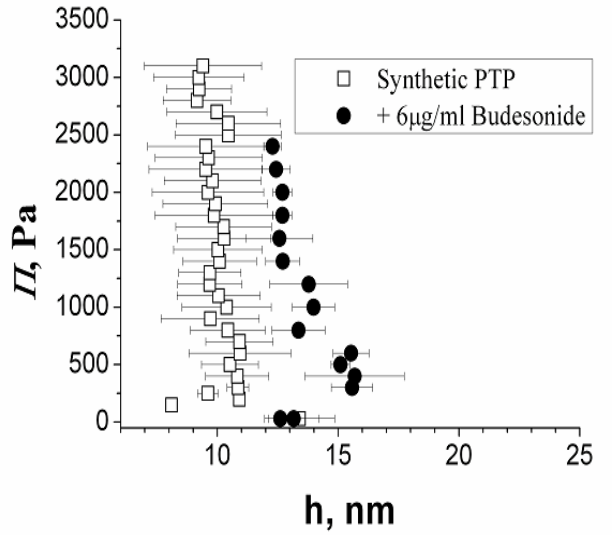

Fig. 4. Disjoining pressure isotherm of synthetic PTP (open symbols) and its mixture with 6 μg/ml Budesonide (filled symbols).

In this case, the isotherms for pure preparation and for mixtures for both corticosteroids shifted to higher thicknesses compared to natural preparation. Film thickness for mixtures was higher than the thickness for pure synthetic PTP while scattering of the results for synthetic preparation was larger.

The shift of isotherms for mixtures of PTP with corticosteroids to higher film thicknesses suggests that a mixed surface layer from PTP and corticosteroids is formed. The isotherms from pure synthetic PTP and its mixtures with corticosteroids have different course in the region below 1500 Pa. The isotherms for mixtures show smooth transition from 15 nm to 12 nm in the region below 1500 Pa, while isotherms for pure synthetic PTP have almost the same thickness, approximately 11 nm.

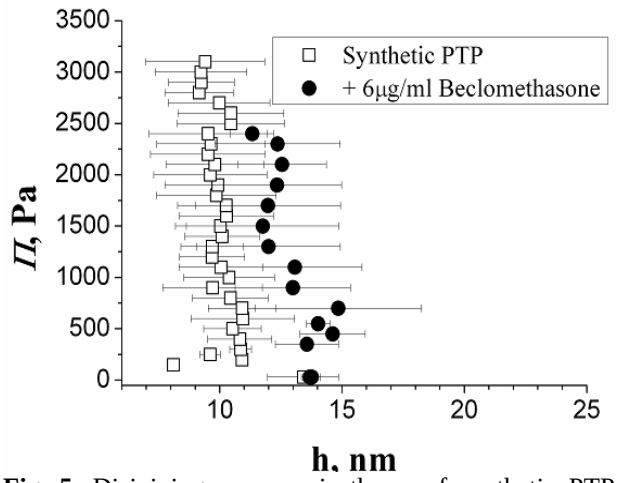

Fig. 5. Disjoining pressure isotherm of synthetic PTP (open symbols) and its mixture with 6 μg/ml Beclomethasone (filled symbols).

Likewise, there is a difference in isotherm course for pure natural PTP and pure synthetic PTP in the region below 1000 Pa. For pure natural PTP the films show smooth thinning from 10 nm to 7 nm. In the same region, the isotherm for pure synthetic PTP shows almost no change in film thickness, approximately 11 nm. It is possible that the smooth thinning observed in the isotherms for pure natural PTP and in isotherms for mixtures and synthetic PTP with corticosteroids, is due to the long electrostatic forces acting in them. Above 1000 Pa, no changes in film thicknesses are observed in the isotherms for pure natural and pure synthetic PTP. As for the mixture of synthetic PTP with 6 μg/ml Budesonide, no changes in film thickness appear above 1500 Pa. These observations may be attributed to the steric forces acting within this range.

In our study we established that at low concentrations Budesonide destabilized thin liquid foam films from Curosurf, while at higher concentrations it stabilized these films. Beclomethasone showed a stabilizing effect at all concentrations studied. This result is in compliance with the observation of Wang et al. [5], namely, that Infasurf may carry less than 1% Budesonide and up to 10% Beclomethasone without significantly compromising its surface activity. Our study was carried out with approximately 3% and 6% of corticosteroids from the weight of the mixture with natural or synthetic PTP. A destabilization of foam films from synthetic PTP in the presence of 3% of Budesonide was observed.

CONCLUSION

The obtained results show that corticosteroids influence formation and stability of pulmonary surfactant foam films. They also suggest that a mixed adsorption layer from pulmonary surfactant studied components is formed in the presence of corticosteroids. This effect is more pronounced in synthetic PTP.

The study shows differences in surface properties of synthetic and natural PTP when Budesonide and Beclomethasone are present, which could be attributed to differences in the formation of the mixed adsorption layer.

The results obtained confirm that the black foam film method is applicable to the study of drug influence on pulmonary surfactant surface activity.

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REFERENCES

1. Zuo Y., R. A. W. Veldhuizen, A. W. Neumann, N. O. Petersen, F. Possmayer, *Biochim. Biophys. Acta*, **1778,** 1947 (2008).
2. C. M. Thorp, Pharmacology for the Health Care Professions, John Wiley & Sons, Chichester, 2008.
3. P. Mikolka, D. Mokra, J. Kopincova, L. Tomcikova-Mikusiakova, A. Calkovska, *Physiol. Res.*, **62,** 191 (2013).
4. E. Lopez-Rodriguez, J. Perez-Gil, *Biochem. Biophys Acta*, **1838,** 1568 (2014).
5. Y. Wang, H. Zhang, Q. Fan, C. Neal and Y. Zuo, *Soft Matter*, **8**, 504, (2012).
6. F. M. de Benedictis, A. Bush, *Am. J. Respir. Crit. Care Med*, **185**, 12 (2012).
7. C. Dani, I. Corsini, S. Burchielli, V. Cangiamila, M. Longini, F. Paternostro, G. Buonocore, F. Rubaltelli, *Pediatr. Pulmonol.*, **44**, 1159 (2009).
8. A . Sato, M. Ikegami, *PLoS One*, **7,** e39392 (2012).
9. R. Cohen, D. Exerowa, *Adv. Colloid Interface Sci.* **134-135,** 24 (2007).
10. D. Exerowa, A. Kruglyakov, Foam and foam films, Elsevier, Amsterdam, 1998.
11. D. Platikanov, D. Exerowa, Thin liquid films. In: Lyklema J. (editor). Fundamentals of interface and colloid science. Soft colloids. Academic Press/Elsevier. Chapter 6, 2005.

ВЛИЯНИЕ НА КОРТИКОСТЕРОИДИ ВЪРХУ ФОРМИРАНЕТО И СТАБИЛНОСТТА НА ТЪНКИ ТЕЧНИ ФИЛМИ ОТ ПУЛМОНАРНИ ТЕРАПЕВТИЧНИ ПРЕПАРАТИ

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(Резюме)

За лечение на възпаление на белите дробове се използват лекарства съдържащи кортикостероиди. Поради амфифилната природа на кортикостероидите, те са повърхностно активни молекули и могат да променят повърхностните свойства на пулмонарния сърфактант. В настоящото изследване, природни и синтетични пулмонарни терапевтични препарати (ПТП), се използват като модел на пулмонарния сърфактант. Влиянието на кортикостероидите върху повърхностната активност на ПТП е изследвано с метода на черния пенен филм . При определянето на вероятността за образуването на черни пенни филми, стабилизирани със синтетични ПТП в присъствието на ниски концентрации на кортикостероиди, се наблюдава дестабилизация на филмите. При високи концентрации филмите се стабилизират. Изотермите на разклинящото налягане показват повишаване на дебелината на филмите от ПТП и кортикостероиди. Резултатите предполагат формирането на смесен адсорбционен слой на пулмонарния сърфактант в присъствието на кортикостероиди. Регистрираният ефект е по-силен при синтетичните ПТП.