Dynamic bubble tensiometry as a useful tool for assessment of physicochemical interactions of liposomal drug carriers with respiratory tract

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This article presents an experimental investigation of physicochemical interactions of novel liposomal drug carriers intended for inhalation with model lung surfactant system. The research was done with the use of maximum bubble pressure (MBP) method to measure changes of dynamic surface tension of pulmonary surfactant system after contact with liposomal colloids made of lecithin and lecithin/cholesterol (3:1 w/w) before and after 5 and 10 minutes of nebulisation by pneumatic nebulizer. What is important to point, MBP method allows the discussion of measured phenomena with reference to the time scales characteristic for the changes of alveolar surface area during the breathing cycle. Obtained results may have a potential application during design and development of new therapeutic liposomal formulations for treatment of respiratory as well as systemic diseases.
Keywords: pulmonary surfactant, liposomes, inhalation, surface tension, adsorption

Introduction

Liposomal drug carriers are one of the most promising and developing strategies of drug administration to respiratory tract, nowadays. Main advantage of their application is the suitability for lipophilic drugs, which facilitates the transport of therapeutic substances to epithelial cells and alveolar macrophages. In addition, drug encapsulation in liposomes prior to administration can result in prolonged drug presence and decreased systemic side effects as compared to systemic route [1].

Pulmonary surfactant is a highly specialized system present in all mammalian lungs, which consists of approximately 90% of lipids and 10% of proteins. It enables the gas exchange surface available during breathing by its surface tension-reducing properties, and represents early lung defence strategies of the host avoiding air- or blood-born pulmonary infection and lung tissue injury [2]. Considering important physiological functions of lung surfactant it is justified to evaluate experimentally possible impact of inhalable liposomal drug carriers on its vital biophysical properties.

Among all known methods, which can be used for measurements of dynamic surface tension in short time range, the maximum bubble pressure (MBP) method is the most appropriate one for medical applications involving biological liquids [3, 4]. What is important to add, dynamic surface tension of biologic liquids is essentially more sensitive to various pathologies than the corresponding equilibrium surface tension values [3-5]. Therefore, dynamic surface tensiograms can be regarded as a comprehensive indicator of some pathologic disturbances, and have a large potential for differential diagnosis and monitoring of the efficiency of therapies [6]. Surprisingly, hardly any works using MBP method in the studies of dynamic interfacial activity of pulmonary surfactant can be found. Considering this, it is justified to dedicate experimental investigation to examine, with the aid of MBP method, the effect of novel liposomal drug carriers intended for inhalation on the dynamic surface activity of lung surfactant.

Materials and methods

In this study, the therapeutic formulation Survanta (Abbott Laboratories, France), being an animal-derived pulmonary surfactant preparation intended for treatment of Respiratory Distress Syndrome in newborn premature infants, was used as a model of pulmonary surfactant. As it was concluded in earlier works (for example [7-8]), Survanta possesses surface activity similar to natural human surfactant in dynamic conditions. Experiments were run in such a way that the concentration of phospholipids in all investigated surfactant formulations was constant (0.75 mg/ml), regardless of the contents of additives. For dilution purposes the sterile isotonic saline (0.9 % w/w NaCl aq.) was applied (Gilbert Laboratories, France).

Liposomal drug carriers were prepared by film hydration method using lecithin (Acros Organics, USA) and lecithin/cholesterol (Sigma Aldrich) mixture (3:1 w/w) dissolved in chloroform (Chempur, Poland). After solvent evaporation in a rounded flask, the lipid film was hydrated with sterile isotonic saline (Gilbert Laboratories, France).

All samples of liposomal colloids used in dynamic measurements with Survanta solutions contained 0.75 mg/ml either lecithin or lecithin/cholesterol as estimated based on deposition of inhaled aerosol formulations. Liposomal colloids were atomised by pneumatic nebuliser RF6 (Flaem Nuova, Italy) connected to MP1 compressor (Medbryt, Poland) for 5 and 10 minutes and then added to model lung surfactant solutions.

Dynamic surface activity of pulmonary surfactant components in investigated systems was studied with the aid of bubble tensiometer BP2 (Krüss, Germany), equipped with a hydrophobised glass capillary (inner diameter of 0.24 mm) and a special testing cell which held only about 5-6 ml of liquid. Thanks to that, considerably smaller amounts of Survanta could be used for every single experiment as compared to standard BP2 cell. Some preliminary research was done with Tween80 solutions of various concentrations (results not published yet) and it was concluded that smaller volume of testing cell had no significant effect on obtained dynamic surface tension results. The BP2 device enabled measurements of surface tension changes during the formation of a fresh air-water interface which is represented by growing air bubble in the tested solution. The time range of surface formation extended from 10 ms to 20-30 s in every single experiment. All experiments were run in triplicate in constant physiological temperature of 37°C assured by the external thermostat.

Results and Discussion

The results of experiments with model pulmonary surfactant and aerosolized liposomal drug carriers are summarized in Figs. 1 and 2 for liposomes consisted of lecithin and lecithin/cholesterol (3:1 w/w) respectively. On each graph there is a comparison of dynamic surface tension profiles of sterile isotonic saline, Survanta solution with phospholipid concentration of 0.75 mg/ml and Survanta solution with lecithin or lecithin/cholesterol mixture content 0.75 mg/ml. Curves are expressed as means with average standard deviation almost in all cases around ±1.0 mN/m (standard deviation not presented on the graphs). Isotonic saline exhibits no surface activity as reflected by a horizontal line in all plots, while Survanta solution - even though at relatively low concentration - shows noticeable surface activity illustrated by a falling surface tension curve.

Considering the impact of nebulised liposomes on the physicochemical properties of the surfactant solution, it is seen that both types of liposomal formulations changed the Survanta tensiograms, i.e. altered the rate and magnitude of adsorption of Survanta surface-active components. Aerosolized lecithin/cholesterol liposomes increased surface tension of surfactant solution especially for shorter surface lifetimes (up ca. 8000 ms). Similar effect was observed for lecithin liposomes. What is interesting, before nebulisation a slight decrease of surface tension was noticed for lecithin liposomes practically in the whole range of surface age, whereas for mixed liposomes such effect appeared only for longer surface lifetimes (from ca. 3000 ms).

For further analysis of dynamic tensiograms obtained from the studies of pulmonary surfactant systems the following parameters were chosen:

γ_1 surface tension at t=0.01 s;

γ_2 surface tension at t=2 s;

γ_3 γ_∞ derived obtained by extrapolation for $t \rightarrow \infty$;

The value γ_1 is characteristic for the properties of the solvent and adsorption processes in the short lifetime range, while the value of γ_2 is characteristic for medium surface lifetimes. These processes represent mostly low and medium molecular weight surfactants in pulmonary surfactant solutions, while the values of γ_3 represent properties of high-molecular compounds. The selection of parameters were done according to suggestions of Kazakov et al. [9] with one modification – for the value of γ_2 the surface lifetime of 2 s not 1 s was chosen as it more precisely represented the characteristic timescale of changes of alveolar surface during the breathing cycle (i.e. the approximate time of inspiration or expiration phase).

The results of calculations with statistical analysis of differences are presented in Table 1. All the parameters are expressed as mean ± standard deviation. Statistical analysis used the Student's *t*-test for comparisons of discrete data points. Differences were considered statistically significant if the probability of the null hypothesis was < 0.05 or less.

In majority of cases, values of surface tension parameters revealed no statistically significant differences between Survanta solution and Survanta solution combined with liposomal colloid. The statistically significant effect was observed for the shortest surface lifetime in case of Survanta mixed with nebulised lecithin liposomes and the relation is dependent on the time of atomisation. In terms of γ_2, which is the parameter representing surface tension for the timescale characteristic for changes of alveolar surface during breathing and because of that particularly interesting

Table 1. Dynamic surface tension characteristics of Survanta +Liposomal colloid solutions in sterile isotonic saline before and after 5 and 10 minutes of nebulisation (Lip. L – lecithin liposomes; Lip. L/C – lecithin/cholesterol 3:1 w/w liposomes).

Substance	Surface tension parameters[a] (average±SD, n = 3)		
	γ_1 [mN/m]	γ_2 [mN/m]	γ_3 [mN/m]
Survanta	69.9 ± 0.6	59.5 ± 1.3	37.4 ± 4.4
Survanta + Lip. L	68.4 ± 1.2	58.0 ± 0.9	38.3 ± 0.4[b]
Survanta + Lip. L 5 min	70.6 ± 0.2[b]	60.5 ± 0.6	37.8 ± 1.4
Survanta + Lip. L 10 min	71.2 ± 0.3[b]	60.0 ± 1.5	27.5 ± 6.0
Survanta + Lip. L/C	70.0 ± 0.3	60.0 ± 1.2	35.8 ± 4.3
Survanta + Lip. L/C 5 min	70.4 ± 1.0	63.0 ± 1.3[b]	37.9 ± 2.5
Survanta + Lip. L/C 10 min	71.2 ± 0.7	63.5 ± 0.8[b]	37.0 ± 2.9

[a] The values of γ_1 represents surface tension at t = 10 ms; γ_2 represents surface tension at t = 2000ms; γ_3 represents surface tension derived from extrapolation for t→∞.

[b] Statistically significant difference between the parameter's value for Survanta solution and Survanta + Liposomal colloid solution.

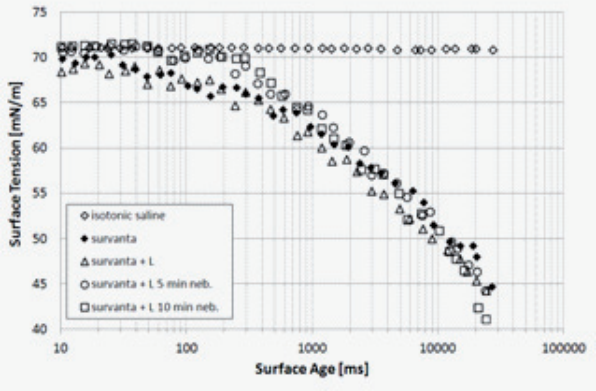

Fig. 1. Dynamic tensiograms of sterile isotonic saline, Survanta solution and Survanta with nebulised 5 and 10 minutes lecithin liposomes (L) at 37°C

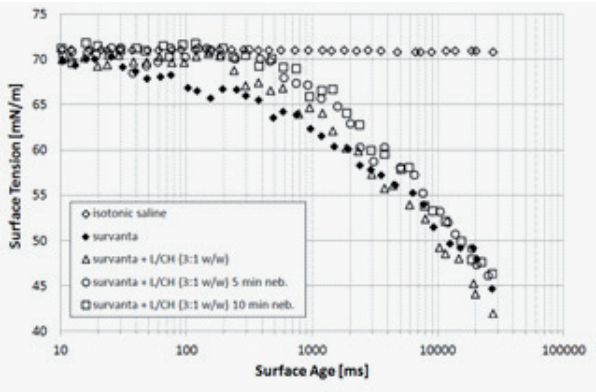

Fig. 2. Dynamic tensiograms of sterile isotonic saline, Survanta solution and Survanta with nebulised 5 and 10 minutes lecithin/cholesterol (3:1 w/w) liposomes (L/CH) at 37°C

in this study, only nebulised lecithin/cholesterol liposomes changed significantly surface activity of Survanta solution. Considering γ_3, an increase of standard deviation values was observed and practically no interaction of liposomal colloids on Survanta dynamic properties for long surface lifetimes characterised by this parameter.

A functional pulmonary surfactant should adsorb rapidly to form a film at the air-water interface of the lungs and thus cause immediate decrease in the surface tension. Any disturbances in the rate and magnitude of adsorption have potentially undesirable effect on the surfactant physiological functions. Mentioned alterations may appear due to compositional changes in surfactant system or functional inactivation via various inhibitors [2], including supraphysiological levels of cholesterol [10] and free fatty acids [11]. Considerable effort has been directed toward studying possible mechanisms of pulmonary surfactant inhibition and several concepts were proposed such as competitive adsorption [12], coagulation of surfactant phospholipids [13] and protein molecules and fluidization of the surfactant phospholipids film [14]; latter two being commonly associated with inhibition by proteins and unsaturated lipids.

Observed in this study slight decrease of Survanta solution surface activity after contact with relatively low-concentrated liposomal colloids could be explained by mentioned above mechanisms. Obtained results indicate that during atomisation vesicle destruction would have occurred and subsequent incorporation at the air-water interface or interaction of free lecithin and cholesterol molecules with surface-active components of model pulmonary surfactant had taken place.

Conclusions

The influence of two types of aerosolized liposomal drug carriers on the physicochemical properties of model pulmonary surfactant was investigated by dynamic bubble tensiometry. For lecithin/cholesterol liposomes mainly negative influence on biophysical activity of Survanta solutions was demonstrated, whereas partially opposite result was observed for lecithin liposomes. However, the effect was statistically significant only in few analysed cases, which may be considered as a positive result, reducing the likelihood of occurring negative side effects during possible future treatment.

This work has demonstrated that while designing new therapeutic formulations intended for delivery by inhalation one has to carefully consider interactions between active substances and pulmonary surfactant. Any deviations in pulmonary surfactant activity may imply a detrimental effect on respiratory physiology, as biophysical properties of lung surfactant correlate with its physiological functions. The proposed approach for in vitro studies with the use of MBP method has proved to be useful, cost-effective and time-saving tool for preliminary assessment of such interactions, which is of great importance while designing new pharmaceutical products. Further research is certainly needed in terms of other medical products as well as more profound analysis of mechanisms of pulmonary surfactant-additive interactions. Possible results can be of significant benefit as they open up the opportunity for development of more effective and safe liposomal therapeutic formulations suitable for drug delivery by inhalation.

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