Biochimica et Biophysica Acta 1838 (2014) 474–481

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Biophysical inhibition of synthetic vs. naturally-derived pulmonary surfactant preparations by polymeric nanoparticles

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ARTICLE INFO

Article history:
Received 7 June 2013
Received in revised form 16 October 2013
Accepted 21 October 2013
Available online 1 November 2013

Keywords:
Biophysical inhibition
Polymeric nanoparticles
Pulmonary drug delivery
Pulmonary surfactant
Surface activity

ABSTRACT

Reasonable suspicion has accumulated that inhaled nano-scale particulate matter influences the biophysical function of the pulmonary surfactant system. Hence, it is evident to provide novel insights into the extent and mechanisms of nanoparticle–surfactant interactions in order to facilitate the fabrication of safe nanomedicines suitable for pulmonary applications.

Negatively- and positively-charged poly(styrene) nanoparticles (diameters of ~100 nm) served as model carriers. Nanoparticles were incubated with several synthetic and naturally-derived pulmonary surfactants to characterize the sensitivity of each preparation to biophysical inactivation. Changes in surface properties (i.e. adsorption and dynamic surface tension behavior) were monitored in a pulsating bubble surfactometer.

Both nanoparticle formulations revealed a dose-dependent influence on the biophysical behavior of all investigated pulmonary surfactants. However, the surfactant sensitivity towards inhibition depended on both the carrier type, where negatively-charged nanoparticles showed increased inactivation potency compared to their positively-charged counterparts, and surfactant composition. Among the surfactants tested, synthetic mixtures (i.e. phospholipids, phospholipids supplemented with surfactant protein B, and Venticute®) were more susceptible to surface-activity inhibition as the more complex naturally-derived preparations (i.e. Alveofact® and large surfactant aggregates isolated from rabbit bronchoalveolar lavage fluid).

Overall, nanoparticle characteristics and surfactant constitution both influence the extent of biophysical inhibition of pulmonary surfactants.

1. Introduction

Nanomedicine represents a valuable drug delivery platform for the treatment of lung disorders following inhalation [1]. Among the numerous potential carrier systems, polymeric nanoparticles (NP) enable controlled drug release and targeting properties and thus, optimized the pharmacokinetic [2–4] and dynamic [5,6] profile of the encapsulated drug within the respiratory tract. However, the safety assessment of nano-scale drug delivery vehicles is currently a subject of intense research [7,8], especially after pulmonary challenge [9–11]. So far, evidence has accumulated that physicochemical (i.e. size and surface charge) and material (i.e. degradability) properties of polymeric NP as well as their concentration at the target site determine inflammatory responses within the lung [12,13]. Another toxicological aspect of lung-delivered NP arises from their direct interaction with the pulmonary surfactant system, a research field where only scant information is available [14–18].

Pulmonary surfactant, a mixture of ~90% of lipids (mainly phospholipids (PL)) and ~10% of proteins (mainly surfactant associated proteins (SP)), that covers the alveolar region of the lung prevents collapse of the alveoli by a drastic reduction in surface tension and concurrent promotion of the gaseous exchange [19,20]. A complex interaction between PL and SP enables the formation of films highly enriched with PL at the air–water interface [21]. During surface film compression (expiration), SP aid to purify the monolayer by removing the less surface active components into the bulk phase [22]. Upon inspiration (expansion of the alveolar surface area), SP facilitate a rapid re-entry and re-spreading of surfactant compounds located in the bulk phase.

Abbreviations: BAL, bronchoalveolar lavage; BALF, bronchoalveolar lavage fluid; c_{NP}, concentration of polymeric nanoparticles; DLS, dynamic light scattering; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; γ_{ads}, surface tension after film adsorption; γ_{min}, minimum surface tension during film oscillation; IC_{50}, half maximal inhibitory nanoparticle concentration; LDA, laser Doppler anemometry; LSA, large surfactant aggregates; MWCO, molecular weight cut-off; NP, nanoparticles; p, probability value; PA, palmitic acid; PBS, pulsating bubble surfactometer; PDI, polydispersity index; PL, phospholipids; PLM, phospholipid mixture; PLM-B, phospholipid mixture supplemented with surfactant protein B; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol); PS, poly(styrene); PS100-, negatively-charged poly(styrene) nanoparticles (nominal diameter: 100 nm); PS100+, positively-charged poly(styrene) nanoparticles (nominal diameter: 100 nm); S.D., standard deviation; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SP, surfactant associated protein; rSP, recombinant surfactant associated protein; TEM, transmission electron microscopy

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http://dx.doi.org/10.1016/j.bbamem.2013.10.016

Hence, factors affecting surfactant biophysical characteristics might have severe outcome [15]. Pulmonary surfactant function can be dramatically influenced by particulate matter as demonstrated for inorganic (i.e. gold [23,24], titanium dioxide [25], and hydroxyapatite [26]), composite (i.e. AmorSil [27,28]) and polymeric (i.e. gelatin [29–31], poly(n-butyl cyanoacrylate) [32], poly(styrene) [25,33], poly(lactide-*co*-glycolide) [33] and EUDRAGIT® E100 [33]) NP. However, it remains challenging to provide definite conclusions on the extent of surfactant inhibition by NP from the existing in vitro studies, due to the employment and combination of diverse NP and pulmonary surfactant (i.e. synthetic [23,24,27–32] and naturally-derived [24–26,33]) preparations and experimental setups (i.e. film balance [24,26–32], captive- [23] and pulsating bubble surfactometer (PBS) [25,33]).

Therefore, this study aimed at characterizing biophysical interactions between lung surfactants and two standardized polymeric NP formulations to provide systematic insights into the NP-surfactant interplay. NP were characterized for size, size distribution, morphology, and ζ-potential by dynamic light scattering, transmission electron microscopy, and laser Doppler anemometry. Next, the surface properties of the synthetic and naturally-derived pulmonary surfactants with or without polymeric NP were examined by monitoring the equilibrium (γ_{ads}) and dynamic (γ_{min}) surface tension behavior in a PBS. Finally, the extent of biophysical inhibition was analyzed from the obtained dose-effect curves (e.g. half maximal inhibitory NP concentration value (IC_{50})). We hypothesized that the sensitivity of the respective pulmonary surfactant preparation to biophysical inhibition by polymeric NP is significantly affected by both the NP characteristics and surfactant composition.

2. Materials and methods

2.1. Materials

Negatively- and positively-charged poly(styrene) NP with nominal diameters of 100 nm (PS100− and PS100+) were purchased from Polysciences (Eppelheim, Germany) and Invitrogen (Darmstadt, Germany), respectively. Alveofact® was obtained from Lyomark (Oberhaching, Germany). Venticute® was from Nycomed (Konstanz, Germany). 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (sodium salt) (POPG) and palmitic acid (PA) were acquired from Sigma-Aldrich (Steinheim, Germany). All other chemicals and solvents used in this study were of the highest analytical grade commercially available.

2.2. Methods

2.2.1. Preparation and characterization of NP

PS100− and PS100+ were purified by dialysis against distilled water (MWCO: 50,000 Da, Spectra/Por® 6, Breda, Netherlands) to remove additives (i.e. surface active stabilizers and preservatives). The actual NP concentration in suspension was assessed gravimetrically after lyophilization (ALPHA 1–4 LSC, Christ, Osterode, Germany). The particle size (i.e. hydrodynamic diameter) and size distribution (i.e. polydispersity index (PDI)) of NP were measured by dynamic light scattering (DLS), and their ζ-potential was determined by laser Doppler anemometry (LDA) (Zetasizer NanoZS/ZEN3600, Malvern Instruments, Herrenberg, Germany). The morphology of NP was investigated by transmission electron microscopy (TEM) (JEM-3010 TEM, JEOL, Eching, Germany).

2.2.2. Isolation of SP-B

SP-B was isolated from Alveofact® by means of LH60-chromatography as described previously [34–36]. The purity amounted to 95% as assessed by SDS-PAGE [36,37]. The concentration of the purified SP-B was determined using a protein assay [38].

2.2.3. Bronchoalveolar lavage (BAL) and isolation of large surfactant aggregates (LSA) from BAL fluid (BALF)

Male rabbits (body weight: 2.5–3.5 kg) were sacrificed by intravenous application of a lethal dose of pentobarbital/ketamine. A catheter was immediately placed into the trachea and lungs were lavaged three times with 50 ml of ice-cold isotonic saline. After filtration through sterile gauze and sedimentation of cells (300 *g*, 15 min, 4 °C), supernatants were pooled and stored at −80 °C until further processing. LSA were isolated from BALF by high speed centrifugation (48,000 *g*, 60 min, 4 °C; Sorvall centrifuge (SS34 rotor), DuPont, Bad Homburg, Germany) [39,40].

2.2.4. Determination of PL content

Lipids were extracted from the surfactant preparations according to Bligh and Dyer [41]. PL were quantified by means of a colorimetric phosphorus assay [42].

2.2.5. Preparation of surfactant materials for biophysical studies

A synthetic PL mixture (PLM) was prepared by dissolving DPPC (69.0 wt.%), POPG (22.0 wt.%) and PA (9.0 wt.%) [43] in a mixture of chloroform/methanol (2/1 (v/v)) followed by sample drying under nitrogen gas. Supplementation of the PLM with SP-B (PLM-B) was achieved by adding the hydrophobic SP dissolved in chloroform/methanol to the organic PLM stock solution before drying [36]. Venticute® and Alveofact® were supplied as lyophilized powders, while LSA was isolated by high-speed centrifugation from rabbit BALF. All surfactant preparations were adjusted to a final PL concentration of 50 mg/ml.

2.2.6. Biophysical studies

Surface activity of samples was assessed by the oscillating bubble technique using a PBS (Electronetics Corp., Amherst, USA) as previously described [33,44]. The technique provided read-outs of the surface tension after film adsorption (γ_{ads}, static measurement) and at a minimum bubble radius during film oscillation (γ_{min}, dynamic measurement). Measurements of the prepared surfactants were performed at a constant PL concentration of 2 mg/ml in isotonic NaCl solution containing 2 mM Ca^{2+} at 37 °C (samples were fabricated from pulmonary surfactant stock suspensions). The concentration of polymeric NP (c_{NP}) added (from polymeric NP stock suspensions) to the surfactant preparation was adjusted between 0.2 and 5 mg/ml prior to the surface activity experiments. Briefly, after a 30 min incubation period at 37 °C (without shaking), samples of 30 μl were transferred to the disposable sample chamber, and adsorption rate was measured. Therefore, a bubble of minimal radius (0.4 mm) was created and while maintaining the bubble at that minimal size without pulsation, pressure difference across the air/liquid interface was monitored. Next pulsation was started by sinusoidally oscillating the bubble radius between 0.4 and 0.55 mm. The cycling rate was set to 20 cycles/min. The pressure differences across the air/liquid interface were recorded continuously. Using the Young–Laplace equation, the surface tension was calculated with a microprocessor. γ_{ads} and γ_{min} values were read after 12 and 300 s, respectively. Dose-effect curve characteristics were calculated using a sigmoidal dose-response function (GraphPad Prism 5, GraphPad Software, La Jolla, USA).

2.2.7. Statistics

All measurements were carried out in triplicate and values are presented as the mean ± S.D. unless otherwise noted. To identify statistically significant differences, one-way ANOVA with Bonferroni's post *t*-test analysis was performed (SigmaStat 3.5, STATCON, Witzenhausen, Germany). Probability values of $p < 0.05$ were considered significant.