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Analysis of the structure and surfactant activity of novel formulations containing exogenous pulmonary surfactant and glucocorticoids

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ABSTRACT

Exogenous pulmonary surfactant (EPS) could be used as carrier of glucocorticoids (GCs) in therapy for respiratory diseases. We formulated novel combination drug products containing bovine EPS and one GC (10 wt%): beclomethasone (Be), budesonide (Bu) or fluticasone (Flu), and studied the GCs action on the surface activity and biophysical properties of EPS.

Subtype ratio was evaluated by phospholipid determination; surface tension (ST) with a pulsating bubble surfactometer and conformational changes by Electron Spin Resonance (ESR).

GCs were incorporated into EPS in more than 80%. None of them generated disaggregation of surfactant, only Bu was found in the light subtype. Bu and Be caused minimal changes in fluidity on polar region of bilayers, but these changes were not enough to inactivate the surfactant. Flu did not significantly alter any biophysical properties or surface activity.

These novel combination EPS-GC products might be a promising strategy in the therapy of pulmonary diseases as the incorporation of the GCs tested did not cause detrimental effects on EPS functionality.

1. Introduction

Chronic lung disease can be caused by several alterations that damage the lung tissue and are often associated with inflammatory processes that alter the normal function of pulmonary surfactant (PS). PS is a lipid–protein material that coats the entire mammalian respiratory surface. It forms a surface-active film at the air-water interface of alveoli, capable of reducing surface tension to near 0 mN/m to prevent pulmonary collapse during expiration and to minimize the work required for inhalation (Creuwels et al., 1997). Although PS composition varies among different species and environmental conditions, it is mainly made up of phospholipids (80–90%), mostly saturated dipalmitoyl-phosphatidylcholine (DPPC), neutral lipids (6–10%) of which cholesterol (Cho) is the most abundant; and at least four specific proteins (5–10%), two hydrophilic (SP-A and SP-D), with immune function, and two hydrophobic (SP-B and SP-C), which contribute to the mechanical stability of the interfacial film and are essential for surfactant activity.

The administration of exogenous pulmonary surfactants (EPS) often provide immediate relief of symptoms and improve oxygenation and gas exchange in some of chronic lung diseases (Günther et al., 2001; Poulain and Clements, 1995; Veldhuizen et al., 1996). At physiological temperature, two phases coexist in monolayers and bilayers of almost all lung surfactants: a semi-crystalline liquid-ordered phase (Lo) and a liquid-disordered phase (Ld) (Alonso et al., 2004; de la Serna et al., 2004). This coexistence of phases is crucial for the surfactant activity, and the role of Cho in this lateral organization has been extensively researched. Several studies have revealed that Cho at 5–10 wt% has no negative effects on EPS function, but that supra-physiological levels of this compound are detrimental (Gunasekara et al., 2005; Palmer et al., 2000; Zhang et al., 2012; Zuo et al., 2008). Although the mechanism of Cho-induced inhibition has not been fully elucidated, many groups, including our own, have demonstrated that the presence of this compound causes alterations of the microstructure of EPS films with the subsequent inactivation of the surfactant (Keating et al., 2007; Leonenko et al., 2007; Malcharek et al., 2005; Martínez Sarrasague et al., 2013). For this reason some authors recommend avoiding the presence of cholesterol in EPS formulations (Yu and Possmayer, 1994).

Glucocorticoids (GCs), due to their anti-inflammatory actions, have been commonly used to modify the course of chronic lung

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disease (Jobe, 2009; Shah et al., 2012). Over the years, the use of systemic corticosteroids has been replaced for topical administration of GCs directly to the lungs, because of their serious side effects (Cole, 2000). Two different pulmonary drug-delivery methods have been clinically tested: inhalation delivery of steroid aerosols and, most recently, intra-tracheal instillation of steroids using EPS as a carrier (Nimmo et al., 2002; Wang et al., 2012; Yang et al., 2010). Many authors have demonstrated that the intratracheal co-administration of EPS and GCs such as budesonide (Bu) or beclomethasone (Be) significantly improved pulmonary outcome in meconium aspiration or respiratory distress syndromes, showing that these substances have a certain therapeutic synergism: the surfactant improves respiratory mechanics and the GCs reduce the inflammation (Dani et al., 2009, 2011; Kuo et al., 2010; Mikolka et al., 2013; Yang et al., 2010). However, despite their low risk-benefit ratio, the pulmonary co-administration of GCs and EPS requires a comprehensive understanding of the molecular interaction between them.

All corticosteroids are biochemically derived from Cho and hence share a close structural similarity with it (Ghosh et al., 1996). For this reason GCs might modify the properties of the surfactant and lead to its inactivation, similarly to Cho. Only few studies have assessed the GC-EPS interaction and there is disagreement about the possible deleterious effect of these compounds on the surfactant, depending on the EPS studied and on the amount of GCs added. Yeh et al. (2008) reported that the dynamic surface activity of the suspension was minimally affected when Bu was added to Survanta at 2 wt%, but when it was added at 25 wt%, this GC blocked the ability of Survanta to reduce the surface tension (ST). On the other hand, Palmer et al. (2000) found that the addition of Bu at low (0.6 wt%) and high (20 wt%) concentration adversely affected the surface-tension properties of two Cho-containing surfactant preparations (Survanta and BLES), meanwhile Zhang et al. (2012) demonstrated that Bu (10 wt%) had no deleterious effect on a Cho-free surfactant preparation (Curosurf).

It is known that the local lung delivery efficiency for drugs in general and GCs in particular, is very low (e.g. between 4.4% and 26.6% for budesonide aerosolized), and depends on the system utilized (Berlinksi and Waldrep, 1997).

As Hidalgo et al. (2015) discussed in their review, diverse strategies have to be developed to improve the delivery of active molecules into airways. Among them, the pulmonary surfactant could be considered a promising strategy for transporting drugs efficiently. It provides advantages because it can dissolve and transport poorly water-soluble drugs along the entire respiratory surface, while avoiding the physiological barriers of the air pathway. Since the GCs are highly lipophilic, it could be thought that GCs and EPS formulated together, with the GC incorporated into the membranes of the EPS, could be a novel alternative for pulmonary drug-delivery. This combination drug product would allow a more efficient delivery of the GCs in the alveolar region of the lung, due to its lipid composition and spreading capabilities. We have not found studies with this type of formulation in the literature. In such preparations, EPS not only act as a carrier but could be expected to have therapeutic effect by itself. Therefore, it is critical that the GCs incorporated to EPS do not impair its biophysical properties and allow the desired synergistic effect.

In order to investigate whether GCs incorporated into surfactant membranes modify the structure of EPS and alter the surfactant activity we carried out the present study. To achieve these objectives, we prepared a combination drug product containing an exogenous surfactant (Prosurf) and one of the GCs commonly used in pulmonary therapy: beclometasone, budesonide and fluticasone (Flu) and analyzed the surfactant macrostructure, the bilayer organization and their relation with the surfactant activity.

2. Materials and methods

2.1. Samples

2.1.1. Exogenous pulmonary surfactant (EPS)

Prosurf is an active pharmaceutical ingredient (API) produced at industrial scale in Argentina (Nialtec S.A., Buenos Aires, Argentina). This API has been used by the pharmaceutical industry (GeMePe SA and Richet SA laboratories) for the elaboration of therapeutic surfactants. Prosurf is a sterile chloroform solution containing surfactant lipids and lipophylic proteins from broncho alveolar lavage fluid of bovine lungs (Hager and De Paoli, 2001). Prosurf is composed of: phospholipids (PL) 94.8%; DPPC46% of total PL; Cho 4.4% and proteins (SP-B, SP-C) 0.8%. Chloroform was evaporated at low pressure and below 40 °C; the pellet was resuspended in sterile saline solution (0.9% NaCl) at 50 °C obtaining a final PL concentration of 30 mg/ml. This final suspension, fractionated in sterile vials, constitutes the exogenous pulmonary surfactant (EPS). EPS was diluted with saline solution (0.9% NaCl) to a final PL concentration of 10 mg/ml and pH5.8–6.0, and this diluted EPS was used as control.

2.1.2. Combination drug product (EPS-GC)

EPS with the different GCs (EPS-Be, EPS-Bu and EPS-Flu) was performed as follows: an appropriate amount of each GC in chloroform solution was added to Prosurf in order to obtain a GC/PL weight ratio 1:10. Then, chloroform was evaporated and the preparation of EPS continued as is detailed above (2.1.1).

Adequate aliquots of cholesterol chloroform solution were added to Prosurf (before solvent was evaporated) in order to obtain EPS with extra Cho (10 wt%) in equivalent proportions to GCs. This sample (EPS-Cho) was used as positive control.

All samples were diluted with saline solution to a final PL concentration of 10 mg/ml and final pH5.8–6.0.

2.2. Chemicals

Budesonide and cholesterol were purchased from Sigma. Fluticasone propionate (Sigma) was donated by Casasco Laboratory, and Beclomethasone dipropionate was purchased from Saporiti SACIFIA. The spin derivatives of stearic acid, 5- and 16-doxyl stearic acids (5DSA and 16DSA respectively) were purchased from Sigma. All the reagents were of analytical grade.

2.3. Chemical determinations

Phospholipid concentrations were measured by the Stewart (1980) method. Cholesterol was determined by the enzymatic method (Allain et al., 1974).

To determine the concentration of GC in the EPS-GC and in the surfactant sub-fractions, an aliquot of each sample was dissolved in chloroform – methanol (2:1) and its absorbance was measured at 250 nm using a Shimadzu double beam spectrophotometer. The concentration of each GC was calculated using the extinction coefficient from the respective calibration curve.

2.4. Heavy and light subtypes

2.4.1. Isolation

The surfactant subtypes were obtained by centrifugation at 10,000*g* for 20 min at room temperature. The supernatants containing the light subtype were separated, and the pellets with the heavy subtype were washed and resuspended to initial volume with saline solution (0.9% NaCl).