Effect of budesonide and salbutamol on surfactant properties

D. PALMER,1 S. SCHÜRCH,1 AND J. BELIK2

1Respiratory Research Group, and 2Department of Pediatrics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 2T9

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Palmer, D., S. Schürch, and J. Belik. Effect of budesonide and salbutamol on surfactant properties. J Appl Physiol 89: 884–890, 2000.—The objective of this study was to evaluate the in vitro effect of budesonide and salbutamol on the surfactant biophysical properties. The surface-tension properties of two bovine lipid extracts [bovine lipid extract surfactant (BLES) and Survanta] and a rat lung lavage natural surfactant were evaluated in vitro by the captive bubble surfactometer. Measurements were obtained before and after the addition of a low and high concentration of budesonide and salbutamol. Whereas salbutamol had no significant effect, budesonide markedly reduced the surface-tension-lowering properties of all surfactant preparations. Surfactant adsorption (decrease in surface tension vs. time) was significantly reduced (P < 0.01) at a high budesonide concentration with BLES, both concentrations with Survanta, and a low concentration with natural surfactant. At both concentrations, budesonide reduced (P < 0.01) Survanta film stability (minimal surface vs. time at minimum bubble volume), whereas no changes were seen with BLES. The minimal surface tension obtained for all surfactant preparations was significantly higher (P < 0.01), and the percentage of film area compression required to reach minimum surface tension was significantly lower after the addition of budesonide. In conclusion, budesonide, at concentrations used therapeutically, adversely affects the surface-tension-lowering properties of surfactant. We speculate that it may have the same adverse effect on the human surfactant.

INHALED DRUGS ARE ROUTINELY utilized in neonates with respiratory conditions. Two of the most commonly used agents are budesonide as a glucocorticoid therapy for the prevention and treatment of chronic lung disease and salbutamol as a bronchodilator.

The clinical efficacy of these inhaled drugs when used in infants is controversial. Whereas some clinical studies have demonstrated acute bronchodilation after salbutamol administration to mechanically ventilated premature newborns and infants (14, 21, 22), other studies showed either no significant change (20) or worsening (38) in the infants’ lung mechanical properties. Similarly, budesonide was shown to be safe and effective as an anti-inflammatory inhaled agent in infants with asthma by some investigators (2, 33, 36) but not others (35), and its usefulness in the prevention of bronchopulmonary dysplasia in neonates has been disputed (9, 23).

Primarily in the case of a bronchodilator such as salbutamol, factors such as the absence of bronchoconstriction (4) or the type of aerosol delivery system utilized (13, 14, 16) may explain the conflicting reports of its efficacy. Yet the possible deleterious effect of these drugs on the surfactant biophysical properties, when administered by inhalation, has not been previously studied.

The surface-tension properties of surfactant have been previously shown to be adversely affected by inhaled environmental toxins (7, 11, 18) and at least one drug used therapeutically (17). In addition, premature neonates may be more susceptible to the harmful effects of inhalant agents on the surfactant, given their low-surfactant pool and the possible interference of aerosol agents with exogenously administered surfactant. The later is a consideration, given that the use of inhaled anti-inflammatory agents has been suggested for the prevention of chronic lung disease in infants as young as 3 days of age (5).

Thus the purpose of the present study was to evaluate in vitro the changes in surface-tension properties after the addition of budesonide and salbutamol, which are exogenous surfactant preparations commonly utilized in neonates, and a natural rat lung lavage surfactant. We hypothesized that budesonide and/or salbutamol would adversely affect the surfactant biophysical properties.

MATERIALS AND METHODS

Surfactant Preparations

Artificial preparations. Two commercially available bovine surfactant preparations were utilized: bovine lipid extract surfactant (BLES) and Survanta. These preparations were obtained by generous donations of BLES Biochemicals (London, Ontario) and Ross Laboratories (Columbus, OH), respectively.

BLES is isolated by organic extraction of bovine lung lavage material. The resulting surfactant preparation contains all of the phospholipids of natural surfactant and the two small hydrophobic proteins, surfactant protein (SP)-B and SP-C; all of the SP-A has been removed. The preparation

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was supplied in an unbuffered normal saline (pH 5–6) with a phospholipid concentration of 27 mg/ml.

Survanta contains phospholipids, including disaturated phosphatidylcholine, triglycerides, neutral lipids, free fatty acids, and surfactant-associated proteins SP-B and SP-C to which colfosceril palmitate [1,2-dipalmitoyl-sn-3-glycero phospholipid choline (DPPC)], palmitic acid, and tripalmitin are added to standardize the composition and to mimic surfactant concentrations of 1 mg/ml of phospholipid used in the captive bubble system give results consistent with the di-
face-tension-lowering properties of natural lung surfactant. The solution was supplied as an 8-ml single-dose vial at a concentration of 25 mg/ml of phospholipid suspended in 0.9% sodium chloride solution.

Survanta contains 84% phosphatidylcholine and up to 6% each of free fatty acid and triglycerides and a relatively low amount of SP-B, <0.2% by mass, relative to the lipids. BLES contains ~92% phospholipids, and, in contrast to Survanta, BLES contains >1.5% SP-B by mass of lipids.

Natural surfactant. Natural surfactant was extracted from the lungs of adult Sprague-Dawley rats (male and female) by using three aliquots of lavage fluid for each lung adjusted to the total lung volume. The lavage fluid was a buffered salt solution composed of 140 mM NaCl and 10 mM HEPES at pH 6.9. The lavage from three lungs was pooled and centrifuged at 500 g to remove cells and then at 60,000 g for 1 h to bring down a cell-free pellet. This pellet was suspended in 1.5 ml of 140 mM NaCl-10 mM HEPES-2.5 mM CaCl₂, pH 6.9. An aliquot of 0.5 ml of this sample was used to determine the total phospholipid content. The sample phospholipid concentration was then adjusted to 1 mg/ml by adding the above-mentioned buffered salt solution containing 2.5 mM CaCl₂.

Surfactant concentration. For the experiments, the artificial and natural surfactant preparations were dried and reuspended in 0.9% NaCl-1.5 mM CaCl₂ such that the phospholipid concentration for both preparations in the samples was 1 mg/ml. Generally a phospholipid concentration of 27 mg/ml. Surfactant concentration was meant to reflect the lower reported estimated aero-
sion-expansion process.

Inhaled Drugs

Budesonide (Astra Laboratories) was supplied as a 0.5 mg/ml solution. Salbutamol (Pharma Science) was supplied at a concentration of 5 mg/ml solution. The solvent for both drugs was normal saline.

On the day of the experiment, one of the study drugs and the surfactant preparations were mixed fresh. Given the variability in the rate of lung delivery for salbutamol and budesonide reported in the literature utilizing the different aerosol delivery systems (13–15, 34) and the dose-related efficacy of these drugs (16, 32), we chose to study a low- and high-drug-to-surfactant concentration. The low concentration was meant to reflect the lower reported estimated aero-
sol lung delivery dose for these inhalants. Lung deposition rates varying from 3.1 to 23.4% have been reported for salbutamol (12), and aerosol delivery efficiency between 4.4 and 26.6% has been reported for budesonide (3), depending on the system utilized. The high concentration was chosen to mimic the maximal delivery doses utilized in children (2, 23, 38). In this manner, budesonide and salbutamol were studied at low- and high-drug-to-surfactant concentrations of 6.3 and 200 and 60 and 2,000 µg/ml, respectively. To maintain the surfactant phospholipid concentration constant (1 mg/ml), the volume of the diluent was adjusted based on the volume of salbutamol or budesonide added to the mixture.

Control measurements were obtained by evaluating both surfactant preparations without additives. Six experiments for each of the surfactant-inhalant drug combination were obtained.

Measurement of the Surfactant Biophysical Properties

We utilized the captive bubble surfactometer previously reported by our laboratory (26–30). Briefly, the captive bub-
ble method is an in vitro technique developed for the deter-
mation of the surface tension, area, and volume of a bubble with a surfactant film at the air-water interface of the bubble. Changing the pressure within the closed chamber can alter the bubble size and thus the surface tension of any insoluble film at the bubble surface.

The instrument consists of a sample chamber constructed from cylindrical glass tubing. A metal piston with a tight O-ring seal is fitted into the glass tubing. The ceiling of the chamber consists of a slightly concave surface of a 1% agarose gel cylinder attached to the metal piston, generating a completely hydrophilic surface for contact with the bubble. The temperature of the chamber was maintained at 37°C and filled with the suspension to be investigated, and a 6-mm-diameter bubble was introduced. The chamber content (~1 ml) was stirred with a small magnetic bar.

Calculation of surface tension, area, and volume. The bubble was recorded continuously throughout the experiment on a video recorder (Sony EVO-9800A recorder and Pulnix TM-7EX camera). Surface tension, area, and volume were calculated from video images of the bubble height and diameter (26). Minimum surface tension was obtained when the bubble ceased to flatten and began to decrease in its width on small compression steps.

Adsorption measurements. Initially, a bubble was intro-
duced into the chamber through a small inlet at the bottom of the chamber. The bubble moves up through the surfactant solution and comes to rest at the agarose surface. The moment the bubble comes to rest and assumes a Laplacian shape was considered to be time 0. Adsorption was assessed by measuring the decrease in surface tension over time.

Quasi-static isotherms. After adsorption to the equilibrium surface tension of ~25 mN/m, the bottom inlet of the bubble chamber was sealed, and the film at the bubble air-liquid interface was compressed stepwise with a pause at each step until the bubble shape no longer changed noticeably within 20–30 s (this corresponds to a change in surface tension of <0.5 mN/m in that period). Approximately 10 steps were taken for each compression and expansion part of the cycle. Four consecutive quasi-static cycles were conducted on each chamber filling. Quasi-static is used here in accordance with physiological manipulations employed for pressure-volume relations of excised lungs. Quasi-static cycling involves a series of small, discrete alterations in bubble area where the surface film is allowed to partially relax during the compres-
ion-expansion process.

Film stability. After four quasi-static cycles were com-
plicated, a fifth quasi-static compression was conducted to
achieve minimum surface tension. The bubble was then held at minimum volume, and the surface-tension increase with time was monitored for 5 min.

**Film area compression.** The film area compression required to reach minimum surface tension was calculated from the film area at the surface tension of ~25 mN/m (equilibrium) and that at minimum surface tension. The area compression is expressed as a percentage of the surface area at 25 mN/m. The area compression required to reach a minimum surface tension is indicative of the composition (quality) of the surface film (25, 30).

**Statistical Analysis**

Data were evaluated by two-way ANOVA. Multiple comparisons between the budesonide and salbutamol data at different time periods were analyzed by the Newman-Keuls multiple-comparison test. Data are reported as means ± SE.

**RESULTS**

Significant changes in the surface-tension properties of both artificial surfactant preparations were observed in the presence of budesonide but not salbutamol. The adsorption for BLES and Survanta measured as surface-tension changes over time is depicted in Fig. 1. The addition of budesonide decreased film adsorption, as seen in the significantly increased surface-tension values for the two artificial surfactant preparations. These adsorption changes were present at the high-budesonide concentration in BLES and at both concentrations in Survanta.

Budesonide adversely affected the surfactant film stability at minimum surface tension at low and high concentrations in the Survanta but not in the BLES preparation (Fig. 2). The minimal surface-tension values increased significantly with the addition of budesonide at a low concentration in the BLES preparation and at a high concentration in the Survanta preparation (Fig. 3). The film area compression was significantly altered by the addition of budesonide at low and high concentrations in Survanta and at high concentration in the BLES preparation (Fig. 4).

Figure 5 depicts the fourth quasi-static isotherms for the Survanta preparation after the addition of budesonide. These isotherms were significantly altered at both concentrations of budesonide (P < 0.01). No significant changes were observed when budesonide was added to BLES.

To confirm that the budesonide effect on the surfactant biophysical properties was not unique to the artificial preparations, we conducted similar experiments with a natural surfactant obtained from adult rat lung lavage. Budesonide at a low concentration (37.8 μg/ml) resulted in an increase in minimal surface tension from 1.46 ± 0.05 to 2.08 ± 0.04 mN/m (P < 0.01) and film area compression from 21 ± 4 to 37 ± 6% area change (P < 0.05). Natural surfactant film adsorption was also adversely affected by the addition of this low concentration of budesonide (Fig. 6).

Experiments were also conducted in the captive bubble system by using only budesonide and salbutamol, both at their high concentration, to assess whether or not these substances alone exhibited surface activity. The addition of budesonide suspension to 0.9% NaCl resulted in an equilibrium surface tension of ~60

Fig. 1. Time course for adsorption of bovine lipid extract surfactant (BLES; A) and Survanta (B) before and after addition of low and high concentrations of salbutamol (Sal) and budesonide (Bud). Values are means ± SE. **P < 0.01 compared with the respective pure surfactant preparation data (control) by ANOVA and Newman-Keuls multiple-comparison test.
mN/m at 37°C, not far below the surface tension of water, which is ~70 mN/m at 37°C. The addition of salbutamol to saline resulted in a surface tension of ~40 mN/m.

**DISCUSSION**

We have shown that budesonide adversely affected the surface-tension properties of two artificial bovine and natural rat surfactant preparations. Surfactant adsorption, minimal surface tension, film stability, and percentage of area compression were adversely affected by the addition of budesonide at low and high concentrations in either Survanta or BLES. Similarly, in the natural rat surfactant, the minimal surface tension, film adsorption, and area compression were adversely affected after budesonide exposure. The addition of salbutamol had no significant effect on the surfactant biophysical properties of the studied artificial preparations.

To evaluate the potential deleterious effect of these two inhaled drugs in humans, we elected to conduct in vitro studies utilizing two commercially available bovine surfactant preparations. These surfactant preparations, albeit distinct from human surfactant due to the absence of SP-A and SP-D, can significantly lower surface tension when administered to infants with surfactant deficiency (19). No suitable human surfactant preparation is presently available for similar testing, but a rat surfactant was utilized to confirm the findings in a natural surfactant in which all the surfactant proteins are present.

We elected to expose the surfactant preparations to budesonide and salbutamol at concentrations within the range possibly observed when these drugs are clinically used. For that purpose we chose a high concentration similar to the maximum total amount of

Fig. 2. Surface stability observed in surface tension vs. time recording for BLES (A) and Survanta (B) before and after addition of low and high concentrations of salbutamol and budesonide. Values are means ± SE. **P < 0.01 compared with the respective pure surfactant preparation data (control) by ANOVA and Newman-Keuls multiple-comparison test.

Fig. 3. Minimal surface tension obtained for BLES (A) and Survanta (B) before and after addition of low and high concentrations of salbutamol and budesonide. Values are means ± SE. **P < 0.01 compared with the respective pure surfactant preparation data (control) by ANOVA and Newman-Keuls multiple-comparison test.
drug administered to infants as therapeutically recommended. The low concentration was chosen to be in the range of the doses estimated to be delivered to the lung in infants when clinically prescribed. The salbutamol concentration, albeit 10- to 20-fold higher than the average dose administered by aerosol to infants (13), is still within the range reported in the literature for clinical use (38). Despite this rather high concentration, no significant abnormalities were observed for any of the surfactant biophysical properties evaluated. In contrast, the chosen budesonide high concentration is similar to the average dose administered to pediatric patients (6). Significant changes in the surface-tension properties of both surfactant preparations were seen in the present study after a budesonide dose equivalent to <5% of the clinically recommended one.

For the assessment of the surfactant surface-tension properties, we utilized the previously reported captive bubble surfactometer method. This approach provides for accurate measurement of changes in surface tension of natural surfactant preparations, as previously reported by our group (25, 28, 30). Experiments with pulmonary surfactants have shown that, on mechanical compression of the surfactant film, there is preferential squeeze out of non-DPPC lipids (10). Interference with this process may account for the observed changes in film adsorption, minimal surface tension, and ability to compress after the addition of budesonide to the surfactants.

There is evidence that surfactant is adversely affected by inhalant agents. Griese et al. (17) have previously shown that the addition of amphotericin B to natural bovine surfactant preparations (Survanta was one of the studied preparations) significantly altered surfactant surface activity. Other studies addressing acute and/or chronic environmental exposure to toxins have shown similar adverse effects on surfactant surface properties, indicating that inhaled agents can adversely affect the surfactant system (7, 11, 18, 24).

The mechanism by which budesonide adversely affects the surfactant biophysical properties is not clear. A very moderate decrease in surface tension of the water-air interface was seen when budesonide was added to the aqueous subphase in the captive bubble system at 200 μg/ml. The sterol molecule budesonide, having a ring structure with a certain degree of unsaturation and branching, appears to possess the capacity to interfere with the structure of the surfactant monolayer at the air-liquid interface, similar to the surfactant sterol cholesterol. Cholesterol adsorbs poorly to an air-water interface and at 37°C has a moderate effect on surfactant phospholipid adsorption (37). Furthermore, the sterol budesonide may interact with DPPC.
by packing into the cavities between the DPPC fatty acid chains, like cholesterol (31). This would interfere with the DPPC molecules’ packing, thus preventing the surfactant films from reaching near zero minimum surface tension on film compression. This concept is in keeping with our observation of the detrimental effect of budesonide on the surface-tension-lowering capacity, including higher minimum surface tensions and larger film area compressions required to reach these minimal tensions. Lastly, the effect of budesonide on Survanta was greater than that on BLES. This difference might be related to the higher content of SP-B in BLES.

Budesonide is a commonly used inhaled glucocorticoid agent. The present study suggests that inhaled budesonide may interfere with surfactant surface-tension properties and result in significant alterations in its activity. In this manner, the anti-inflammatory, beneficial effect of the drug may be counteracted by its detrimental acute effect on surfactant (natural or exogenous) and consequently result in worsening lung mechanics. The present study was not designed to assess in vivo lung mechanical changes after budesonide administration. Nevertheless, we speculate that the lack of consistent data to support the efficacy of this inhaled glucocorticoid in preventing or ameliorating the clinical course of chronic lung disease in neonates may be related to its adverse effect on surfactant. Lastly, the infants’ age may also have an impact on the in vivo effect of the drug in the lung. Recently beclomethasone, another inhaled corticosteroid, was evaluated in infants within the first 3 days of life for the prevention of bronchopulmonary dysplasia (5). Such early use of inhaled corticosteroids when the surfactant pool of the premature infant is not normal may have a more deleterious effect on the lung mechanical properties than when utilized later in the first month of life.

We are not aware of any reported clinical studies where lung mechanical properties were evaluated in infants immediately after a budesonide inhaled treatment. In contrast, several reports have documented changes in pulmonary function measurements after salbutamol therapy. The drug has either been reported to be a beneficial bronchodilator (8, 21), to have no effect (4), or even to worsen the lung mechanical properties (38) when utilized in infants. Further studies geared to obtaining lung function measurements after budesonide inhalation in infants are warranted based on the presently reported in vitro changes in surfactant biophysical properties.

In summary, we observed a significant impairment of artificial and natural surfactant biophysical properties on exposure to budesonide at concentrations similar to the ones reached in the lower airways when utilized therapeutically. We speculate that this drug may have a negative effect on human surfactant when it is administered to neonates. This work was supported by grants from the Medical Research Council of Canada and the Alberta Heritage Foundation for Medical Research.

REFERENCES


