

Spreading kinetics of the exogenous porcine surfactant Surfacen® mixed with drugs used in respiratory clinics

Odalys Blanco¹, Rebeca Pérez¹, Yuliannis Lugones¹, Andrés Morilla², Roberto Faure¹

¹ Centro Nacional de Sanidad Agropecuaria, CENSA
San José de las Lajas, Mayabeque, Cuba

² Hospital Materno Infantil Dr. Ángel Arturo Aballí
Calzada de Bejucal s/n, Km 7½. Arroyo Naranjo, La Habana, Cuba
oblanco@censa.edu.cu, odalysbh@infomed.sld.cu

REPORT

ABSTRACT

The specific biophysical properties of the surfactant complex, able to adsorb with high efficiency to the air-liquid interface and, through it, quickly travel to the distal airways, becomes the surfactant a potential vehicle for drug delivery by the inhaled route in the treatment of several lung diseases. However it is vital that drugs delivered via lungs did not interfere with the pulmonary surfactant lining layer surface activity. The aim of this study was to evaluate the *in vitro* effect of N-acetylcysteine, hydrocortisone and amikacin on the interfacial property (spreading) of Surfacen®. The interfacial property of porcine lipid extract, Surfacen®, was evaluated *in vitro* by Langmuir balance. Measurements were obtained before and after the addition of a low and high concentration of drugs. The drugs do not affect the ability of spreading Surfacen. Future studies are necessary to evaluate all Surfacen biophysical properties in the presence of these drugs.

Keywords: Surfacen, pulmonary surfactant, N-acetylcysteine, amikacin, hydrocortisone, spreading kinetics

Biotecnología Aplicada 2016;33:1521-1525

RESUMEN

Cinética de esparcimiento del surfactante porcino exógeno Surfacen® mezclado con fármacos empleados en la clínica respiratoria. Las particulares propiedades de biofísicas del surfactante pulmonar, capaces de adsorberse con muy alta eficiencia a la interfase aire-líquido y, a través de ésta, viajar rápidamente a las vías aéreas distales, convierte al surfactante en un potencial vehículo para la administración de fármacos por la vía pulmonar en el tratamiento de varias enfermedades pulmonares. Sin embargo es de vital importancia que el fármaco suministrado vía pulmonar no interfiera con la actividad de superficie del surfactante pulmonar. El objetivo del estudio fue evaluar el efecto de N-acetilcisteína, Hidrocortisona y Amikacina en la propiedad interfacial (cinética de esparcimiento) del surfactante pulmonar, Surfacen®. La propiedad interfacial del extracto lipídico porcino, Surfacen® se evaluó *in vitro* en la balanza de Langmuir, las mediciones fueron obtenidas antes y después de la adición de concentraciones bajas y altas de los diferentes fármacos. La adición de la N-acetilcisteína, Hidrocortisona y Amikacina no afecta la cinética de esparcimiento de Surfacen®. Los fármacos no afectan la capacidad de esparcimiento de Surfacen®. Futuros estudios son necesarios para evaluar todas las propiedades biofísicas de Surfacen® en presencia de estos fármacos.

Palabras clave: Surfacen, surfactante pulmonar, N-acetilcisteína, amikacina, hidrocortisona, cinética de esparcimiento

Introduction

Pulmonary surfactant is a lipoprotein complex essential to allow a stable opening of the pulmonary respiratory spaces, and its absence or dysfunction is associated to the development of severe diseases [1].

A functional pulmonary surfactant should be adsorbed rapidly to form a film at the air-water interface of the lungs, probably within a few seconds, thus lowering the surface tension at very low values on dynamic compression. There are two surface pressure values that have traditionally been used to characterize the functional capacities of the pulmonary surfactant: the surface pressure reached when forming an interfacial film on equilibrium and the surface pressure when the film is subjected to compression, which physiologically occurs at the end of expiration.

Beyond its primary action, the specific biophysical properties of the surfactant complex, able to adsorb with high efficiency to the air-liquid interface and quickly traveling through it to the distal airways, makes the surfactant a potential vehicle for drug delivery by the inhaled route. The integration of drugs

(antibiotics, anti-inflammatory and antioxidants) and the surfactant may allow an efficient quasi-topical administration, with greatly reduced side effects. However, it is vital that drugs delivered via the lungs did not interfere with the surface activity of the pulmonary surfactant lining layer [2-4].

The formulation of multiple drugs inhaled opens up exciting the therapeutic opportunities for treating asthma and chronic obstructive pulmonary diseases (COPD). For instance, Adi *et al.* [5] demonstrated the feasibility of formulating a solution-based pressurized metered dose inhaler containing a triple therapy with identical deposition pattern for the treatment of several respiratory diseases where multi-drug cell targeting is required.

Corticosteroids, because of their anti-inflammatory actions, have commonly been used to modify the course of chronic lung disease [6-8]. However, the use of systemic corticosteroids has adverse effects. In efforts to overcome the adverse effects of systemic corticosteroids, significant attention has been given to

1. Moreno OV, López ML, Dieppa FD, López MAP, Abad AA, Rivero GJ, et al. Estudio de la eficacia del surfacén en el distress respiratorio del recién nacido. *Rev Cubana Pediatría*. 1999;71(2):60-71.

2. Palmer D, Schurch S, Belik J. Effect of budesonide and salbutamol on surfactant properties. *J Appl Physiol* (1985). 2000;89(3):884-90.

3. Yeh TF, Lin HC, Chang CH, Wu TS, Su BH, Li TC, et al. Early intratracheal instillation of budesonide using surfactant as a vehicle to prevent chronic lung disease in preterm infants: a pilot study. *Pediatrics*. 2008;121(5):e1310-8.

4. Zhang H, Wang YE, Neal CR, Zuo YY. Differential effects of cholesterol and budesonide on biophysical properties of clinical surfactant. *Pediatr Res*. 2012;71(4 Pt 1):316-23.

the topical administration of corticosteroids directly to the lungs. Two different pulmonary drug-delivery methods have been clinically tested: inhalation delivery of steroid aerosols [9, 10] and, very recently, the intratracheal instillation of steroids, using exogenous surfactant as a carrier [3, 11-13]. Therefore, it is critical that corticosteroids delivered via the respiratory system did not impair the biophysical properties of the pulmonary surfactant.

In neonatal pneumonia, pulmonary surfactant surface activity tends to be damaged by microorganisms that can invade through the air-liquid interface, so the direct application of antibiotics to the airways offers potential advantages in the treatment and prevention of pneumonia. Despite the high antibiotic dose delivered to the lung, the question of efficacy remains controversial. Explanations for this include failure of the antibiotic to reach the infected lung area. In this sense, the use of surfactant as carrier of antimicrobial agents to deliver them into the lung parenchyma has been more promising and offers an alternative for critically ill patients with pneumonia [14, 15]. One of them, amikacin, is commonly used together with beta-lactam antibiotics to the sepsis treatment in neonates.

Additionally, there is also increasing evidence that the inflammatory processes of COPD are closely associated to oxidative stress. Proinflammatory cytokines and growth factors stimulate the production of reactive oxygen species. N-acetylcysteine (NAC) is a glutathione precursor with beneficial properties. NAC is a scavenger of free radicals as it interacts with reactive oxygen species, such as hydroxyl radical (OH^\cdot) and hydrogen peroxide (H_2O_2). In COPD patients, NAC has been used as a mucolytic and for its antioxidant properties [16, 17].

Considering all these, no previous studies addressed the drug-carrier properties of Surfacen®, a registered, natural exogenous surfactant widely used in Cuba to treat pre-term babies at risk or already suffering neonatal respiratory distress [4]. In this report, the spreading kinetics of Surfacen® was investigated heading for its future use as drug carrier for antimicrobials or hydrocorticosteroids. The effect of three drugs (NAC, hydrocortisone and amikacin) on the interfacial properties of Surfacen® was determined by the equilibrium surface pressure (π) method. This characterizes the spreading kinetics as the maximum surface pressure that an interfacial film reaches spontaneously in the absence of external compression, when phospholipids molecules at the air-water interface are in a thermodynamic equilibrium with the molecules in the bulk phase.

Materials and methods

Surfactant preparation

Surfacen® was supplied by the Centro Nacional de Sanidad Agropecuaria (CENSA, Mayabeque, Cuba). It is obtained from organic extracts of porcine bronchoalveolar lavages and provided as a sterile white lyophilized powder, dosed in 50 mg phospholipid vials [18]. To reconstitute Surfacen® dry powder, the proper amount of the surfactant is weighted and suspended in distilled water, with 60 min preincubation

of the suspension at 37 °C, under shaking at 550 rpm in a thermo-shaker (TS 100, Fisher, USA).

Drugs

NAC was supplied as a 100 mg/mL nebulizer solution (Quimefa, Cuba), hydrocortisone (HCORT; Vitrofarma, S.A.; Bogota DC, Colombia) was supplied as a 100 mg lyophilized powder and amikacin (AMK) was supplied as 500 mg (Quimefa, Cuba).

Surfacen® was experimentally tested at a 15 mg/mL concentration, and NAC and Amikacin at 1 and 10 % regarding the phospholipids concentration, respectively. HCORT was used at 0.1, 5 and 10 %. The surfactant-drug combinations were incubated for 60 min at 37 °C under at 550 rpm in a thermo-shaker (TS 100, Fisher, USA).

Spreading assays in a modified Langmuir balance

Surfacen® suspensions or surfactant-drug combinations were applied with a syringe onto the interface of a specially designed T-shaped Teflon trough (Nima Technology, Coventry, UK) with 15 mL of subphase buffer 5 mM Tris, pH 7.0, plus 150 mM NaCl. Samples were applied in one end of the trough, while a pressure sensor monitored the time-dependent spreading and the lateral diffusion of the surface active material arriving at the other end. Spreading isotherms were obtained at 37 ± 1 °C.

Statistical analysis

Statistical analysis was performed using the SPSS 21.0 software. Data were expressed as mean \pm standard deviation and analyzed by one way ANOVA with least significant difference (LSD) *post hoc* analysis. A probability value of $p \leq 0.05$ was considered statistically significant.

Results and discussion

Effect of NAC, hydrocortisone and amikacin on Surfacen® spreading properties

The π -t spreading kinetics of Surfacen® suspensions and Surfacen®-NAC combinations at the interface, at 37 °C are shown in Figure 1. Surfacen® adsorbed very effectively at the interface, producing an equilibrium surface pressure of approximately 42 mN/m in less than a minute. These results are in agreement with prior evidences obtained by our group [19]. The addition of NAC at 1 % did not affect the Surfacen® spreading kinetics. A different effect was detected at 10 % NAC, with a fast adsorption, followed by a significantly lower pressure ($p \leq 0.05$) after 25 seconds than that obtained with Surfacen® alone. But after 5 min, this combination behaved as Surfacen®. NAC showed no interfacial behavior at the two concentrations tested.

Figure 2 shows the π -t spreading kinetics of Surfacen® suspensions and the three Surfacen®-HCORT (0.1, 5 or 10 %) combinations at the interface at 37 °C. The addition of different concentrations of HCORT to Surfacen® did not affect the spreading kinetics, with HCORT lacking interfacial behavior alone at either concentration.

For amikacin (Figure 3), the π -t spreading kinetics values of Surfacen® suspensions and Surfacen®

5. Adi H, Young PM, Traini D. Co-deposition of a triple therapy drug formulation for the treatment of chronic obstructive pulmonary disease using solution-based pressurised metered dose inhalers. *J Pharm Pharmacol.* 2012;64(9):1245-53.

6. Nannini LJ, Poole P, Milan SJ, Holmes R, Normansell R. Combined corticosteroid and long-acting beta(2)-agonist in one inhaler versus placebo for chronic obstructive pulmonary disease. *Cochrane Database Syst Rev.* 2013;11:CD003794.

7. Shah SS, Ohlsson A, Halliday HL, Shah VS. Inhaled versus systemic corticosteroids for the treatment of chronic lung disease in ventilated very low birth weight preterm infants. *Cochrane Database Syst Rev.* 2012;5:CD002057.

8. Yang CF, Lin CH, Chiou SY, Yang YC, Tsao PC, Lee YS, et al. Intratracheal budesonide supplementation in addition to surfactant improves pulmonary outcome in surfactant-depleted newborn piglets. *Pediatr Pulmonol.* 2013;48(2):151-9.

9. Cole CH, Colton T, Shah BL, Abbasi S, MacKinnon BL, Demissie S, et al. Early inhaled glucocorticoid therapy to prevent bronchopulmonary dysplasia. *N Engl J Med.* 1999;340(13):1005-10.

10. Shah SS, Ohlsson A, Halliday H, Shah VS. Inhaled versus systemic corticosteroids for the treatment of chronic lung disease in ventilated very low birth weight preterm infants. *Cochrane Database Syst Rev.* 2007(4):CD002057.

11. Dani C, Corsini I, Burchielli S, Cangiamila V, Longini M, Paternostra F, et al. Natural surfactant combined with beclomethasone decreases oxidative lung injury in the preterm lamb. *Pediatr Pulmonol.* 2009;44(12):1159-67.

12. Yang CF, Jeng MJ, Soong WJ, Lee YS, Tsao PC, Tang RB. Acute pathophysiological effects of intratracheal instillation of budesonide and exogenous surfactant in a neonatal surfactant-depleted piglet model. *Pediatr Neonatol.* 2010;51(4):219-26.

13. Dani C, Corsini I, Burchielli S, Cangiamila V, Romagnoli R, Jayonta B, et al. Natural surfactant combined with beclomethasone decreases lung inflammation in the preterm lamb. *Respiration.* 2011;82(4):369-76.

14. Haitsma JJ, Lachmann U, Lachmann B. Exogenous surfactant as a drug delivery agent. *Adv Drug Deliv Rev.* 2001;47(2-3):197-207.

15. Schwameis R, Erdogan-Yildirim Z, Manafi M, Zeitlinger MA, Strommer S, Saueremann R. Effect of pulmonary surfactant on antimicrobial activity in vitro. *Antimicrob Agents Chemother.* 2013;57(10):5151-4.

16. Sadowska AM, Manuel YKB, De Backer WA. Antioxidant and anti-inflammatory efficacy of NAC in the treatment of COPD: discordant in vitro and in vivo dose-effects: a review. *Pulm Pharmacol Ther.* 2007;20(1):9-22.

17. De Backer J, Vos W, Van Holsbeke C, Vinchurkar S, Claes R, Parizel PM, et al. Effect of high-dose N-acetylcysteine on airway geometry, inflammation, and oxidative stress in COPD patients. *Int J Chron Obstruct Pulmon Dis.* 2013;8:569-79.

18. Manzanares D, Díaz E, Alfonso W, Escobar A, Colomé H, Muñoz MC, et al. Surfactante pulmonar porcino. República de Cuba 1995;A 61:35-42 K.

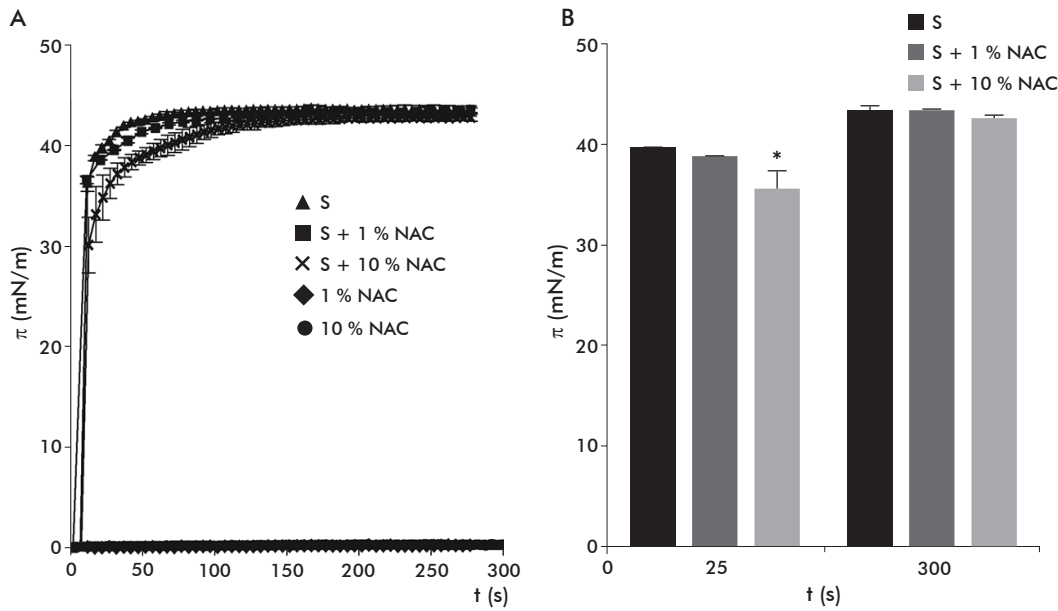


Figure 1. Surfacen® (S) interfacial adsorption and Surfacen® plus N-acetylcysteine (NAC) preparations. π - t isotherms have been obtained upon spreading 150 μ g phospholipid of Surfacen® or Surfacen®-NAC combinations at 1% and 10% NAC in relation to phospholipid concentration, in a modified Langmuir balance thermostated at 37 °C. A) Surface pressure reached by samples at 25 s ($\pi = 25$ s) or 100 s ($\pi = 100$ s) after applying. B). Values are means \pm SD, * $p \leq 0.05$ compared with Surfacen® alone.

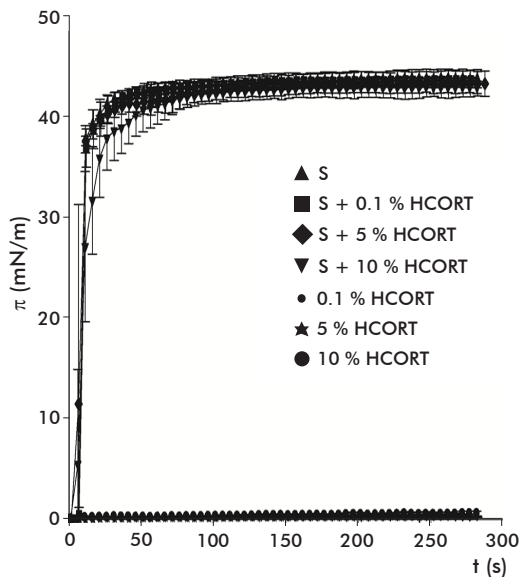


Figure 2. Surfacen® (S) interfacial adsorption and Surfacen® plus hydrocortisone (HCORT) preparations. π - t isotherms have been obtained upon spreading 150 μ g phospholipid of Surfacen® or Surfacen®-HCORT combinations at 0.1, 5 or 10% of HCORT in relation to phospholipid concentration in a modified Langmuir balance thermostated at 37 °C. Values are means \pm SD.

combined with AMIK at the interface, at 37 °C showed that the addition of this antibiotic did not affected the surfactant spreading kinetics. Additionally, amykacin alone showed no interfacial behavior.

Also to evaluate de possible interaction of multiple drug formulations in the spreading kinetics of Surfacen®, the three drugs (NAC, HCORT and amykacin)

were administered together to Surfacen®, and it was demonstrated that their simultaneous administration did not alter the surfactant spreading properties. The mixture of the three drugs at the two concentrations tested showed not any interfacial behavior (Figure 4).

Regarding the biophysical activity of the surfactant, it is determined by three main functions: i) the surfactant must be adsorbed quickly (within seconds) to reduce the surface tension at the interface; ii) the surfactant should be efficiently re-adsorbed at the interface air-subphase (as occurring during inspiration in the alveoli) and, iii) the surfactant must form rigid films during surface compression (as occurring during expiration), which allows to obtain a low surface tension [20].

The pulmonary surfactant should also show a fast interfacial adsorption while equilibrating surface tension to stabilize lungs. During this process, the surfactant proteo-lipid complexes reach and spread into the interface from the sub-phase, forming a surface active interfacial film. The interfacial adsorption process includes both arrival and accumulation of material near the interface, and the ultimate transfer processes that insert the molecules into the interface, forming a layer exposed to air [21]. In this work, the ability to spread Surfacen® after being applied directly to the interface in the presence of the most frequently drugs used in respiratory medicine (an antioxidant-mucolytic, anti-inflammatory and antimicrobial) was evaluated by Langmuir balance.

In another study, the effect of budesonide on the surfactant biophysical properties of two exogenous surfactants (Survanta® and BLES) was evaluated in a captive bubble surfactometer. Another steroid medication, Budesonide, markedly reduced the surface

19. Blanco O, Cruz A, Ospina OL, Lopez-Rodriguez E, Vazquez L, Perez-Gil J. Interfacial behavior and structural properties of a clinical lung surfactant from porcine source. *Biochim Biophys Acta*. 2012;1818(11):2756-66.

20. Schurch S, Green FH, Bachofen H. Formation and structure of surface films: captive bubble surfactometry. *Biochim Biophys Acta*. 1998;1408(2-3):180-202.

21. Lopez-Rodriguez E, Perez-Gil J. Structure-function relationships in pulmonary surfactant membranes: from biophysics to therapy. *Biochim Biophys Acta*. 2014;1838(6):1568-85.

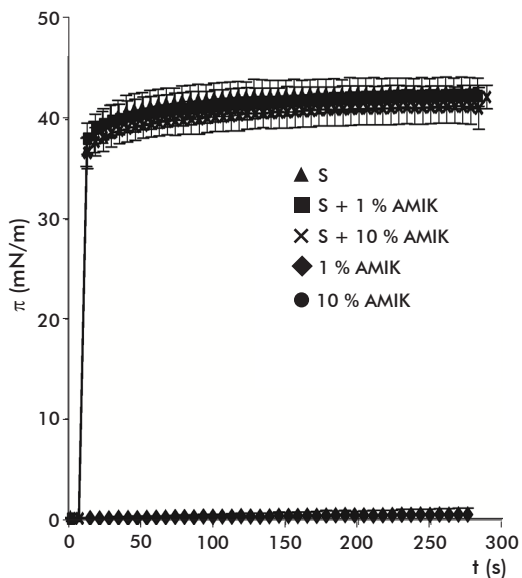


Figure 3. Surfacen® (S) interfacial adsorption and Surfacen® plus amikacin (AMIK) preparations. π - t isotherms have been obtained upon spreading 150 μg phospholipid of Surfacen® or Surfacen®-AMIK combinations at 1 or 10 % of AMIK in relation to phospholipid concentration in a modified Langmuir balance thermostated at 37 °C. Values are means \pm SD.

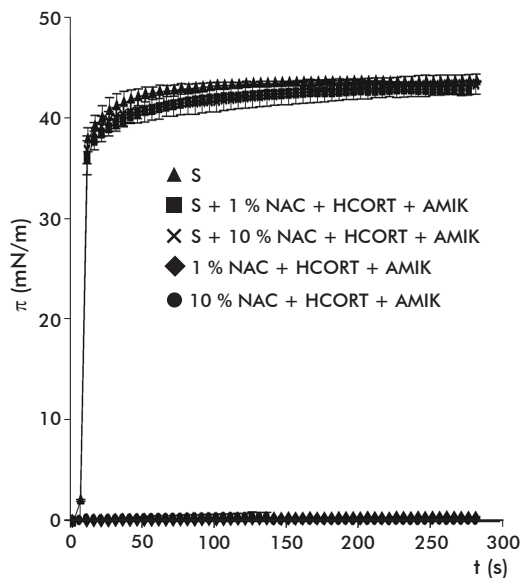


Figure 4. Interfacial adsorption of Surfacen® (S) alone or mixed with N-acetylcysteine (NAC), hydrocortisone (HCORT) and amikacin (AMIK). π - t isotherms have been obtained upon spreading 150 μg phospholipid of Surfacen® or Surfacen® plus NAC, HCORT or AMIK combinations at 1 or 10 % in relation to phospholipid concentration in a modified Langmuir balance thermostated at 37 °C. Values are means \pm SD.

tension-lowering properties of both surfactant preparations [2]. The surfactant adsorption was significantly reduced at a high budesonide concentration with bovine lipid extract surfactant (BLES) and both concentrations with Survanta®. At both concentrations, budesonide reduced Survanta film stability, whereas no changes were observed with BLES. Therefore, it has been concluded that budesonide, at common therapeutic concentrations, adversely affected the surface-tension-lowering properties of surfactant [2].

In contrast, more recently, using a pulsating bubble surfactometer, it was shown that the addition of 2 % Budesonide to Survanta® minimally affected its dynamic surface activity [3]. These authors concluded that budesonide would not reduce the ability of Survanta (*i.e.*, to reduce surface tension) when simultaneously administering it with budesonide at a 50:1 concentration ratio or more. Moreover, they found that the early postnatal intratracheal instillation of budesonide using surfactant as vehicle significantly improved the combined outcome of death or chronic lung disease in small premature infants, without causing immediate adverse effects. Nevertheless, they considered that results should be corroborated in a large sample multicenter clinical trial.

Particularly for corticosteroids, they are biochemically derived from cholesterol and, therefore, they share a close structural similarity with it. Recent findings showed differential effects of cholesterol and budesonide on the biophysical properties of an animal-derived surfactant preparation, Curosurf®. At low concentrations (0.1 or 1 %), neither cholesterol nor budesonide significantly affected the Curosurf® surface activity, and 10 % cholesterol completely

inhibited its surface-tension lowering ability. By contrast, budesonide at the same concentration only moderately reduced film stability and did not significantly alter the compression isotherm [4]. In another research, this author showed that the addition of 10 % budesonide significantly decreased the surface activity of another clinical surfactant preparation, Infasurf® [22]. A main difference between Infasurf® and Curosurf® compositions is that Infasurf® contains 5-8 % cholesterol whereas Curosurf® is cholesterol-free. Remarkably, Surfacen® is a cholesterol-free surfactant preparation and our findings showed that adding hydrocortisone (another corticosteroid) did not affect Surfacen® spreading kinetics, these results consistent with those of previous reports [2-4].

The large surface area for gas exchange made the respiratory system particularly susceptible to oxidative stress-mediated injury. Both endogenous and exogenous pro-oxidants (e.g. cigarette smoke) activate leukocytes and host defenses. Several studies have demonstrated the presence of an increased oxidative stress and decreased antioxidants in subjects with chronic obstructive pulmonary disease (COPD), but the contribution of oxidative stress to the pathophysiology of COPD is generally less discussed. So far, antioxidant drugs such as NAC have been regarded only as mucolytic agents, but several recent clinical trials indicate that NAC may reduce the rate of COPD exacerbations and improve small airways function. The most plausible explanation for the beneficial effects observed in patients with COPD treated with NAC lies in the mucolytic and antioxidant effects of this drug [23, 24]. To our knowledge, there were no previous reports on the evaluation of NAC effects on

22. Wang YE, Zhang H, Fan Q, Neal CR, Zuo YY. Biophysical interaction between corticosteroids and natural surfactant preparation: implications for pulmonary drug delivery using surfactant as a carrier. *Soft Matter*. 2012;8(2):504-11.

23. Santus P, Corsico A, Solidoro P, Braido F, Marco FD, Scichilone N. Oxidative Stress and Respiratory System: Pharmacological and Clinical Reappraisal of N-Acetylcysteine. *Copd*. 2014.

24. Zhang T, Zhou X. Clinical application of expectorant therapy in chronic inflammatory airway diseases (Review). *Experimental and therapeutic medicine*. 2014;7(4):763-7.

the biophysical properties of the pulmonary surfactant for delivery purposes.

Several studies have evaluated the influence of antibiotics on the biophysical properties of the pulmonary surfactants. For instance, five antibiotics (amphotericin B, amoxicillin, ceftazidime, pentamidine and Tobramycin) were tested for their effects on the surface activity of a bovine surfactant [25]. The minimal surface tension of the antibiotic-surfactant mixtures was comparable to that of the surfactant alone. Nevertheless, when the surfactant function was evaluated in the animal model of respiratory failure induced by lung lavage, PaO₂ levels in the animals receiving ceftazidime-surfactant or pentamidine surfactant were unchanged when compared to the surfactant group. PaO₂ levels in animals receiving the mixtures amphotericin B-surfactant, amoxicillin-surfactant or tobramycin-surfactant significantly decreased as compared to the surfactant group. For tobramycin, it was further found that PaO₂ levels were not affected when NaHCO₃ buffer was used surfactant suspension instead of saline. They concluded that some antibiotics affect the in vivo activity of a bovine pulmonary surfactant [25]. Therefore, before using surfactant-antibiotic mixtures in clinical trials, interactions between the two agents should be carefully evaluated. Calkovska *et al.* [26] showed that the Curosurf® biophysical and

physiological properties were improved by the cyclic amphipathic decapeptide polymyxin B. Recently, the mutual influence of the natural surfactant preparation and three antibiotics (amikacin, cefepime, and colistimethate sodium) were characterized in vitro. It was shown that the addition of amikacin or cefepime to the Suzacrin® surfactant had no significant influence on the surfactant surface-active properties. An obvious reduction of the surface-active properties was confirmed for a surfactant/colistimethate composition [27]. In our experimental setting, Amikacin did not affect the Surfacen® spreading properties, corroborating the results obtained with the surfactant Suzacrin® and amikacin [27].

Our work corroborates that Surfacen® is able to retain good surface activity (spreading kinetic) when mixed with anti-inflammatory, antioxidant and antibiotic drugs. Further studies are required to evaluate all Surfacen® biophysical properties in the presence of these drugs, further supporting its simultaneous use as drug carrier to the lung in the clinical setting.

Acknowledgements

This study was supported by a project from Cuban Health Ministry (1008013). The authors thank to Leyanis Aguiar from Censa for her assistance in reviewing manuscript writing and language usage.

25. van 't Veen A, Gommers D, Mouton JW, Kluytmans JA, Krijt EJ, Lachmann B. Exogenous pulmonary surfactant as a drug delivering agent: influence of antibiotics on surfactant activity. *Br J Pharmacol.* 1996;118(3):593-8.

26. Calkovska A, Some M, Linderholm B, Johansson J, Curstedt T, Robertson B. Biophysical and physiological properties of porcine surfactant enriched with polymyxin B. *Biol Neonate.* 2005;88(2):101-8.

27. Birkun A. Exogenous Pulmonary surfactant as a vehicle for antimicrobials: assessment of surfactant-antibacterial interactions in vitro. *Scientifica.* 2014;2014.