SURFACEN® inhibits the growth of bacteria causing respiratory infections

RESEARCH

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ABSTRACT

One pharmaceutical preparation of exogenous lung surfactant, SURFACEN®, was tested *in vitro* on growth of pathogens involved in lung disease. SURFACEN® at concentration of 1, 10 and 20 mg/mL was incubated with *Streptococcus pneumoniae, Streptococcus agalactiae, Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* in saline solution by 5 h at 37 °C. In the presence of SURFACEN® it was observed a decrease of colony forming units in all evaluated concentrations and from 10 mg/mL a reduction of one logarithm of bacterial growth in all types of bacteria tested was observed. SURFACEN® showing antibacterial effect on grampositive and gramnegative bacteria causing lung disease.

Key words: pulmonary surfactant, SURFACEN®, antibacterial, grampositive bacteria, gramnegative bacteria, pneumonia, Acute Respiratory Distress Syndrome

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RESUMEN

SURFACEN® inhibe el crecimiento de bacterias causantes de infecciones respiratorias. Una preparación farmacéutica de surfactante pulmonar exógena, SURFACEN®, se evaluó en relación con el crecimiento *in vitro* de agentes patógenos involucrados en enfermedades pulmonares. SURFACEN®, a las concentraciones de 1, 10 y 20 mg/mL, se incubó con *Streptococcus pneumoniae, Streptococcus agalactiae, Staphylococcus aureus*, *Klebsiella pneumoniae* y *Escherichia coli* en solución salina durante 5 h a 37 °C. En presencia del SURFACEN® se observó una disminución de las unidades formadoras de colonias en las concentraciones evaluadas. A partir de la concentración de 10 mg/mL se observó una reducción de un logaritmo del crecimiento bacteriano, en todos los tipos de bacterias ensayadas. SURFACEN® mostró un efecto antibacteriano en bacterias grampositivas y gramnegativas causantes de enfermedades respiratorias.

Palabras claves: surfactante pulmonar, SURFACEN®, antibacteriano, bacterias grampositivas, bacterias gramnegativas, neumonía, sepsis, Síndrome de Distrés Respiratorio Agudo

*I*ntroduction

The endogenous pulmonary surfactant is a mixture of phospholipids and specific proteins (SP-A, SP-B, SP-C, SP-D). Its main function is the maintenance of a low surface tension at the air-liquid interface in the pulmonary alveoli, therefore reducing the amount of energy involved in breathing and maintaining lung stability through the prevention of alveolar collapse [1]. In addition to its role as a surface active agent, this substance fulfills a defensive role at the lungs [2].

The successful therapeutic use of a natural bovine pulmonary surfactant, marketed under the name of SURFACTANT TA®, on neonates afflicted from Respiratory Distress Syndrome (IRDS), has been known from the decade of the eighties on the past century [3]. In addition to SURFACTANT TA®, there are currently 5 commercially available exogenous natural surfactants of bovine or porcine origin, known as SURVANTA® (marketed in Japan as SURFACTANT TA®), from Abbot Ltd., Ross Laboratories, US, ALVEOFACT®, from Boehringer Ingelheim, Germany, CUROSURF®, from Chiesi Pharmaceuticals, Italia, INFASURF®, from Forest Laboratories, US, and BLES®, from BLES Biochem, Canada. SURFACEN®, developed at CENSA in Cuba [4], is the latest addition to this range of natural surfactants. Generally, the biochemical composition of these substances is characterized by a high phospholipid content, particularly enriched in phosphatidylcholine and its palmitic acid-saturated derivative, dipalmitoylphosphatidylcholine (DPPC), and a predominance of anionic phospholipids such as phosphatidylglycerol and phosphatidylinositol over other phospholipid species. They have also been shown to contain two hydrophobic proteins known as SP-B and SP-C [1].

Surfactant-related distresses have been described for pulmonary diseases of septic origin such as pneumonia and Acute Respiratory Distress Syndrome (ARDS) [5]. There is a scarcity of published literature on the direct influence of the pulmonary surfactant or the available commercial preparations over bacterial growth, and the few reports that do exist show contradictory results [6, 7]. Furthermore, none of the latter studies this aspect in SURFACEN®. The aim of this work, therefore, is the evaluation of the *in vitro* effect of SURFACEN® on the growth of bacteria known to be causative agents for respiratory infections.

*M*aterials and methods

The pharmaceutical preparation SURFACEN® is a natural surfactant obtained from porcine lung washes. It is formulated as a sterile white lyophilizate, each vial containing 50 mg of phospholipids, 0.3 to 0.7 mg

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of hydrophobic proteins, and 2.03 to 3.03 mg of other lipids [8]. It is manufactured and provided by the National Center for Animal and Plant Health (CENSA). The bacterial strains used in this work were *Streptococcus pneumoniae* ATCC 49619, *Streptococcus agalactiae* ATCC 13813, *Staphylococcus aureus* ATCC 6538, *Klebsiella pneumoniae* ATCC 13883 and *Escherichia coli* ATCC 25922. Bacteria were grown in agar-blood media, incubated at 37 ºC for 24 h and lyophilized at 4 ºC. The identity of all strains was verified by the API system (*Analytical Profile index*, bio-Mérieux, France). For the preparation of the inocula, the different cultured strains were diluted in 0.9% NaCl to a concentration of 3 x 10^8 c.f.u./mL according to the McFarland scale; verifying this value by performing serial 1:10 dilutions and seeding 0.1 mL from dilutions 10^{-5}, 10^{-6}, 10^{-7} into 2 tryptone-soy agar plates (TSA), spreading the mixture with an L-rod and incubating the plates for 24 h at 37 ºC on an inverted position. All the inocula were used within the first hour after preparation, and in order to be considered as valid, colony counts had to be in the range of 20 to 200 c.f.u. [9]. In order to measure bacterial growth by the method of quantitative suspension through dilution and counting, tubes containing 0.9% NaCl, the bacterial suspension in saline solution and SURFACEN® diluted in water (1, 10 and 20 mg/mL) were incubated on an orbital shaker at 90 r.p.m. for 5 h at 37 ºC [7]. Aliquots of 100 mL were taken from each tube, serial 1:10 dilutions were performed in saline solution (down to 10^{-6}), and seeded on blood-agar plates. The colonies were counted after a 24 h incubation at 37 ºC, calculating the original number of c.f.u. per mL on the basis of the dilution being used. Three independent measurements were taken for each point. Since bacterial growth is logarithmic, the logarithm of the colony-forming units ($\log_{10}$ cfu/mL) was calculated and used to express the values of reduction of bacterial growth (RBG) as RCB = $\log_{10}$C-$\log_{10}$S, where C: c.f.u. on the control, and S: c.f.u in the presence of SURFACEN®. An antibacterial effect was considered to be present only if microbial growth was reduced in 1 log, that is, with RCB values higher or equal to 1 [10]. For the statistical analysis the results were expressed as the mean ± the mean standard error (MSE) from 3 independent determinations. The data were statistically evaluated by analysis of variance (ANOVA) and a Duncan test, using the SAS application software, version 8.02 TS level 02MO (1999-2001). Differences were considered to be statistically significant when $p < 0.05$.

Results and discussion

The incubation of the Gram positive bacteria *Streptococcus agalactiae*, *Streptococcus pneumoniae* and *Staphylococcus aureus* with SURFACEN® for 5 h resulted in a decrease in bacterial growth, expressed as an increase in RBG (Figure 1). There is a dose-effect trend for *Streptococcus agalactiae* and *Streptococcus pneumoniae*, and dose-dependent for *Staphylococcus aureus*. A reduction higher than 1 log, expressed as RBG values equal to or higher than 1, was observed at concentrations of 10 and 20 mg/mL. Similarly, the incubation of the Gram negative bacteria *Klebsiella pneumoniae* and *Escherichia coli* with SURFACEN®

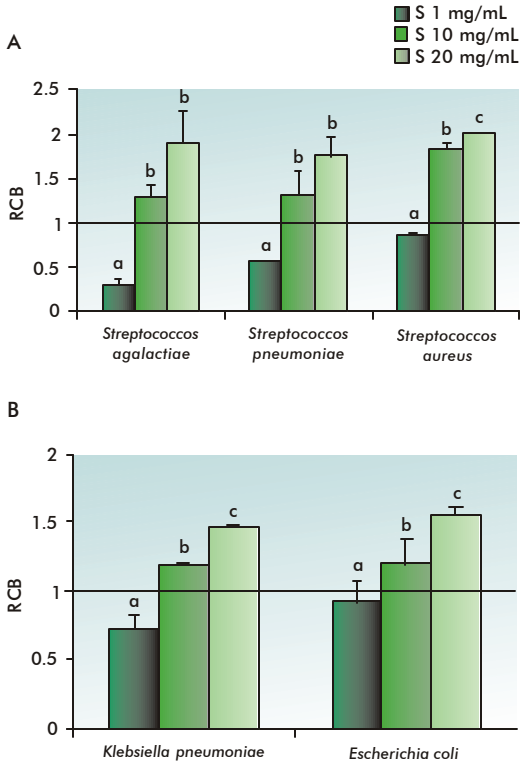

Figure 1. Effect of SURFACEN® on the growth of Gram-positive (A) and Gram-negative bacteria (B). The data are expressed as the mean ± the MSE of the reduction of bacterial growth (RBG). An antibacterial activity was considered as detectable if it reduced microbial growth by at least 1 log, indicated by values higher or equal to 1. Different letters indicate statistically significant differences $p < 0.05$.

resulted in a dose-dependent decrease in the number of colony-forming units, expressed as an increase in RBG (Figure 1B). Bacterial growth decreased 1 log at concentrations of 10 mg/mL and higher of SURFACEN®, and growth was not affected for the microorganisms evaluated in the absence of SURFACEN® (data not shown).

The first evidences of the antibacterial activity of the pulmonary surfactant were obtained with a fraction from the surfactant obtained from rat lung washes that was able to lyse *Streptococcus pneumoniae* and other Gram-positive bacteria such as *Streptococcus viridans*, *Streptococcus pyogenes* and *Streptococcus bovis*. This effect was attributed to the free fatty acids present in the sample [11]. In another report, the bacterial proliferation in lung homogenates decreased in rabbits infected with group B streptococci after treatment with CUROSURF® [12]. It has been also shown that the treatment with this surfactant improves the respiratory function in rats with pneumonia due to *Escherichia coli* [13]. The presence of bactericidal peptides in the lung, such as β-defensins and cathelicidins (for example, LL 37) also contributes to pulmonary defenses [14]. As a matter of fact, the presence of antibacterial peptides on endogenous bovine pulmonary surfactant has been demonstrated [15], and a cathelicidin peptide denominated prophenin due to its high proline and phenylalanine content, together with a C-terminal 18 a.a. fragment from

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prophenin, denominated PF-18, have been detected in porcine pulmonary tissue and in the commercial preparation CUROSURF® [16, 17].

Some studies have been carried out to sort the individual contribution of the components of the surfactant to its antibacterial activity. The hydrophobic protein SP-B has 68% of sequence homology to the peptide antibiotic dermaseptin bI, and is able to inhibit the growth of *Escherichia coli* by itself [18]. Also, it has been shown that phospholipids, and specifically DPPC, can act as inhibitors of the exotoxin produced by β-hemolytic group A and B streptococci [19].

The commercial surfactant preparations have biochemical differences in their composition [20, 21]; and these differences may have a decisive impact on their properties. An evaluation of the performance of 3 surfactant preparations (ALVEOFACT®, SURVANTA® and EXOSURF®) against the growth of *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus agalactiae*, showed that only SURVANTA® inhibited the growth of *Escherichia coli* [6]. In a recent study evaluating the effect of different preparations of exogenous natural (SURVANTA®, CUROSURF®, ALVEOFACT®) and synthetic (EXOSURF® and PUMACTANT®) surfactants on the growth of group B streptococci, *Staphylococcus aureus* and *Escherichia coli*, only CUROSURF® was able to inhibit the growth of group B streptococci, but this effect was not observed against *Staphylococcus aureus* or *Escherichia coli*. None of the surfactants under analysis was able to inhibit the growth of *Escherichia coli*, and SURVANTA®, instead, enhanced the growth of this bacterium [7]. On the other hand, it has been reported that while CUROSURF® has an antibacterial activity against group B streptococci and *Escherichia coli*, the reactive oxygen species released by these bacteria are able to peroxidate the surfactant [22].

Our results constitute the first report on the antibacterial effect of the commercial pharmaceutical preparation SURFACEN® on Gram-negative and Gram-positive microorganisms.

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