An update on clinical surfactant preparations and respiratory disease

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ABSTRACT

The pulmonary surfactant and respiratory disease are tightly linked. Adequate surfactant activity remains critical for optimal lung function throughout life, and secondary surfactant dysfunction may contribute to the process of many lung diseases, including Acute Respiratory Distress Syndrome, asthma, cystic fibrosis and pneumonia among others. Attempts to treat these lung diseases with exogenous surfactants have been only partially successful. The objective of this article is to carry out an update on clinical surfactant preparations and immunomodulatory properties, as well as all related to clinical trials of clinical surfactant preparations and respiratory diseases in the pharmaceutical market today. The results show the potential to extent the use of the exogenous surfactant preparations to other respiratory disease different than Neonatal Respiratory Disease Syndrome. The importance of immunomodulatory functions of lung surfactant and the aim to evaluate the effectiveness of including different drugs as ingredient of a surfactant preparation represent the research's active field on surfactant topic.

Keywords: Pulmonary surfactant, exogenous pulmonary surfactant, clinical pulmonary surfactant, respiratory disease, inflammation

RESUMEN

Una actualizacion sobre las preparaciones clinicas de surfactantes y enfermedades respiratorias. El surfactante pulmonar y las enfermedades respiratorias están estrechamente relacionados. La actividad adecuada del surfactante es esencial para la función óptima del pulmón en los seres humanos. Una disfunción secundaria del surfactante pulmonar puede contribuir al proceso de enfermedades pulmonares como el síndrome de insuficiencia respiratoria aguda, el asma, la fibrosis cística y las neumonías, entre otras. Los intentos de tratar estas enfermedades con surfactantes exógenos han sido parcialmente exitosos. Esta es una actualización referente a las preparaciones clínicas de surfactantes y sus funciones inmunomoduladoras, y lo que acontece en el mercado en relación con los ensayos clínicos que emplean estas preparaciones para mitigar enfermedades pulmonares. Los resultados de un análisis informacional muestran el potencial para extender tales preparaciones en el tratamiento de enfermedades respiratorias diferentes al síndrome de insuficiencia respiratoria aguda del neonato. La relevancia de las funciones inmunomoduladoras del surfactante pulmonar y la evaluación de su potencial formulación de conjunto con otros medicamentos, son un campo de investigación activo.

Palabras clave: Surfactante pulmonar, surfactante pulmonar exógeno, surfactante pulmonar clínico, enfermedades respiratorias, inflamación

Introduction

The pulmonary surfactant is a complex mixture of lipids and proteins, and is synthesized by alveolar type II epithelial cells into space alveolar. It is composed of 90% lipids and around 10% proteins although only 6-8% by mass are specifically surfactant-associated. Phospholipids are about 80-85% of the lipids by weight, being around 75% phosphatidylcholine, 10-15% phosphatidylglycerol plus phosphatidylinositol and less than 5% phosphatidylserine and sphingomyelin. Almost half the content of surfactant phosphatidylcholine fraction is composed of dipalmitoylphosphatidylcholine (DPPC), which is the most abundant lipid but also the main surface-active species in surfactant. Pulmonary surfactant also presents higher proportion of anionic phospholipids (including phosphatidylglycerol and phosphatidylinositol) than most animal membranes. Cholesterol is the main neutral lipid in surfactant, representing 5-10% of the total lipids by mass [1, 2]. In addition to its peculiar lipid composition, pulmonary surfactant contains four specific surfactant-associated proteins named according to their chronological discovery as: SP-A, B, C and D

[3]. SP-A and SP-D are water-soluble proteins while SP-B and SP-C are highly hydrophobic, strongly associated to the surfactant lipids.

The main function of pulmonary surfactant is to reduce the surface tension at the air-liquid interface of the alveolus, avoiding the alveolar collapse and reducing the work of breathing. Besides, its property to reduce the surface tension and physically stabilize the respiratory surface, pulmonary surfactant plays also a major role in the pulmonary defense, preventing the access of pathogens through the large alveolar surface exposed to the environment [4].

The pulmonary surfactant is very important to lung function; therefore, its absence, deficiency or inactivation is associated with severe pulmonary diseases. The therapy with clinical surfactant preparations address to Newborn Respiratory Distress Syndrome (NRDS) was a hit in neonatology and is the treatment of choice in this syndrome until today [5]. The exogenous surfactant was designed to treat a primary inadequacy of surfactant due to lung immaturity; however, there are other respiratory diseases of high incidence

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worldwide [6, 7] such as acute lung injury, acute respiratory distress syndrome (ARDS), pneumonia, bronchopulmonary dysplasia, meconium aspiration, asthma, cystic fibrosis (where the pulmonary surfactant is seriously damaged from the biochemical and biophysical points of view), which in turn will disturb the defensive response in the lung. In this sense, the surfactant preparations used in neonatology has been applied in clinical trials of these diseases but have not been fully effective. Certainly, these pathologies are more complex, taking place inflammatory, infectious and oxidative processes and the reversal of surfactant inactivation being required to obtain surfactant preparations mimicking native lung surfactant (Figure 1). The strategy carried out to get a surfactant preparation more resistant to inactivation is discussed below.

Clinical surfactant preparation

The clinical lung surfactant preparations can be classified in two major groups: 1) modified natural and 2) synthetic lung surfactant. The first one is obtained by bronchoalveolar lavage or mincing of lung and extraction with organic solvents; then, depending on the surfactant, it can be purified by chromatography or supplemented with active ingredients. These surfactant preparations contain all phospholipids present in endogenous lung surfactant; the proteins SP-B and SP-C also form part of its chemical composition, but proteins SP-A and SP-D are not present. The second one is, in turn, divided into a) first-generation protein-free synthetic surfactants composed of phospholipids and chemical agents (detergent o lipids) for adsorption and spreading, which have become unpopular due to their relatively poor clinical performance and b) a new generation surfactant composed of phospholipids and hydrophobic protein which contains synthetic phospholipids and peptide analogues of SP-B and/or SP-C synthesized chemically or obtained in a recombinant step (Table 1). A detailed comparison of lipid and protein compositions of the natural surfactant preparations can be found in a review by Blanco and Perez Gil [8].

Exogenous lung surfactant and immunomodulatory properties *in vitro*

The immunomodulatory activities from clinical lung surfactant have been studied. In table 2, the inmunomodulatory properties of clinical lung surfactant *in vitro* reported until now are summarized. These results support the proposal the clinical lung surfactant is involved in the protection of the alveolar epithelium against the injury caused by reactive oxygen intermediates and pro-inflammatory cytokine and in the down-regulation of inflammatory mediators' production, being mechanistic pathways also proposed [9]. In this sense, these preparations have a potential to be beneficial in reducing the inflammatory reaction in the lungs present in several respiratory disease [8].

What is recently known about the market in relation to clinical trials for clinical lung surfactant preparations?

The search for clinical trials in the Business Insights database (www.business-insights.com), using as search strategy the match of three exogenous
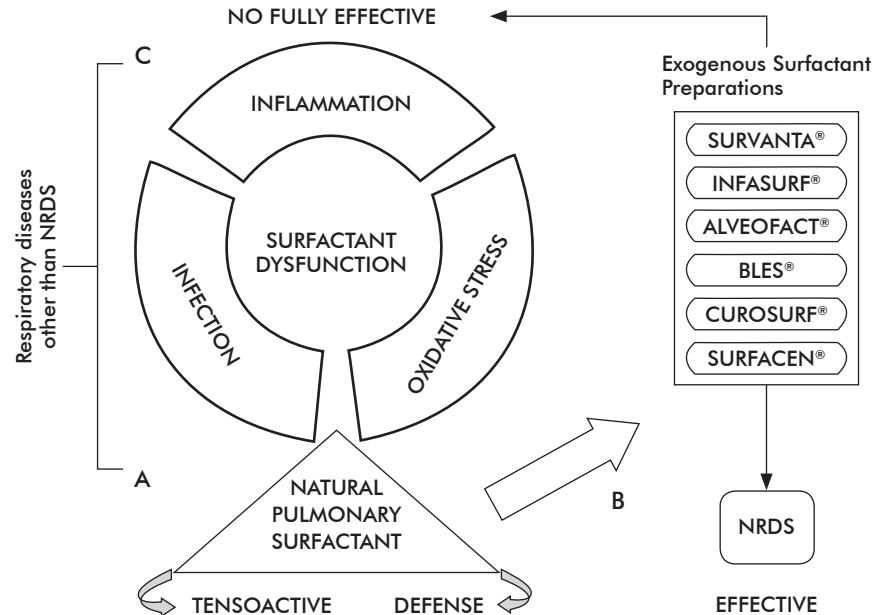

Figure 1. Types of surfactant preparations and their clinical effectiveness. A) Role of lung surfactant in normal lung. B) Use of exogenous surfactant preparations. C) Dysfunction of surfactant by inflammation, oxidative and infection processes to happen in lung injury. NRDS: Newborn Respiratory Distress Syndrome.

(CUROSURF, INFASURF and SURVANTA) and one synthetic (SURFAXIN) surfactant preparations, combined with 'respiratory disease', showed the following results:

CUROSURF®

It has been available in Europe since 1992, where it is the number one selling surfactant according to IMS Health data. Commercialized by eleven companies, among them *Ascent Pharmaceuticals Limited* (formerly Genepharm Australia Ltd), from which it is available in the market. In September 2005, Douglas Pharmaceuticals announced that a new treatment, CUROSURF®, was available in Australia under license. From *Chiesi Farmaceutici S.p.A*, it is available in the pharmaceutical market. On May 7, 2007, data presented at the Pediatric Academic Societies' Annual Meeting demonstrated that premature infants with neonatal RDS have a nearly 20% better chance of survival if they were treated with CUROSURF® instead of the two competing surfactant therapies (INFASURF® and SURVANTA®). On September 25, 2006, CUROSURF®, is the world leading surfactant indicated in premature infants for the prevention and treatment of RDS. From *Torrex Chiesi Pharma GmbH*, Since February 24, 2011, CUROSURF® is intended to be marketed in the territories of Russia and some countries of the Commonwealth of Independent States by *Chiesi*.

INFASURF®

Commercialized by four companies: *Recordati SpA*; *Forest Laboratories Inc.*; *Ikaria Holdings, Inc.* and *Samaritan Pharmaceuticals Inc.* From *Recordati SpA* it is currently in Phase II/III clinical trials indicated to treat RDS. From *Forest Laboratories Inc.*, INFASURF® is available in USA. It was launched

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Table 1. Clinical surfactant preparations being applied in clinical trials

Type of surfactant	Trade name	Composition
Native (as well called exogenous, modified natural or clinical surfactant preparations)*	CUROSURF® / Chiesi Farmaceutici Pharma, Italy	Poractant alfa, a natural pulmonary surfactant consisting of polar lipids and hydrophobic low molecular weight protein. Available as intratracheal suspension (120 mg per 1.5 mL or 240 mg per 3 mL)
	SURVANTA® / Abbott Laboratories, Abbott Park, IL, USA	Contains beractant as the active ingredient, is a sterile intratracheal suspension. It is an intratracheal suspension (25 mg/mL) that is regarded as a surfactant replacement therapy and is indicated to prevent or treat RDS in premature infants
	SURVANTA TA® (SURFACTEN) / Tokyo Tanabe Co, Tokyo, Japan	SURVANTA® Japanese version
	ALVEOFACT® / Boehringer Ingelheim, Germany	A phospholipid fraction obtained by cow-lung lavage. It is indicated for the treatment of RDS in premature infants. Each vial contained a suspension of 50 mg phospholipid per 1.2 mL
	INFASURF® / Forest Pharmaceuticals, Inc, St Louis, MO, USA	Contains calfactant, an extract of natural surfactant from calf lungs which includes phospholipid, neutral lipids, and surfactant protein B (SP-B) and SP-C. It is indicated for the treatment of RDS in premature infants and is available as intratracheal suspension 210 mg of phospholipid in 6 mL
	BLES® / BLES Biochemicals, London, ON, Canada	A phospholipid fraction of cow lung obtained by lavage. It is indicated for the treatment of RDS in premature infants. Each vial holds a suspension containing 27 mg of phospholipids per mL
	SURFACEN® / Censa, Cuba	A natural pulmonary surfactant consisting of phospholipids and hydrophobic low molecular weight protein; available as an intratracheal lyophilizate (50 mg of phospholipid).
Synthetic†	EXOSURF® / Burroughs Welcome, Research Triangle Park, NC, USA	Synthetic surfactant, containing dipalmitoylphosphatidylcholine (DPPC), hexadecanol and tyloxapol
	SURFAXIN® (SINAPULTIDE) / Discovery Laboratories, Warrington, PA, USA	Synthetic surfactant containing lucinactant, which is comprised of a novel KL4 peptide sinapultide, DPPC, sodium palmitoyloleoylphosphatidyl glycerol and palmitic acid. Sinapultide is a 21-amino acid peptide designed to mimic the function of human SP-B
	VENTICUTE®/Byk Gulden, Konstanz, Germany	Synthetic surfactant containing 2% modified human recombinant protein SP-C and DPPC, phosphatidylglycerol, and palmitic acid

* Native surfactants are indicated as treatment for the Respiratory Distress Syndrome (RDS) in premature infants.
† EXOSURF® has currently become unpopular due to relatively poor clinical performance. SURFAXIN® and VENTICUTE® are being evaluated in clinical trials to treat RDS in premature infants.

Table 2. Immunomodulatory properties of clinical surfactant

Clinical surfactant	Immunomodulatory properties	Source
CUROSURF®	Comparison to SURVANTA®. Both preparations decreased the release of elastase induced by interleukin-8 (IL-8), neutrophil-activating protein-2 and formylmethionyl-leucyl-phenylalanine in polymorphonuclear leukocytes	[10]
	Inhibits the *in vitro* synthesis of secretory type IIA phospholipase A(2) in alveolar macrophages and in acute lung injury model in guinea pig	[11, 12]
	Inhibits the production of superoxide anions stimulated with the standard bacterial extract OM-85 but not with phorbol myristate acetate; the release of prostaglandin E2, thromboxane and leukotriene and the release of the tumor necrosis factor-alpha (TNF-α) in a dose-dependent fashion in human monocytes	[13]
	Down regulated CD14 expression and up-regulated CD206 in macrophages, inhibited phagocytosis of *E. coli* and up-regulated mRNA expression of scavenger receptors CD36, CD68, SR-A and LOX-1 in macrophages	[14]
	Comparative study with ALVEOFACT® and VENTICUTE®. CUROSURF® modulated the viability of eosinophils: increased the number of necrotic eosinophils, increased the levels of eosinophil cationic protein and induced chemotaxis against autologous eosinophils	[15]
	Comparison to VENTICUTE®. Inhibited the lipopolysaccharide (LPS)-induced increase in TNF-α expression. A comparison of both preparations revealed a similar effect on IL-10 expression, although this last was higher after incubation with VENTICUTE®. CUROSURF® increased IL-8 expression at higher concentrations, but VENTICUTE® had no effect	[16]
	Down-regulated LPS-induced interleukin-8 expression in A549 cells line at mRNA and protein levels. The mechanism proposed was inhibiting the translocation of Toll-like receptor 4 into membrane lipid raft microdomains induced by LPS	[17]
SURFACTANT TA®	Inhibits adherence and superoxide production in human neutrophils	[18]
SURVANTA®	Inhibits the recombinant interferon gamma-induced production of tumor necrosis factor-alpha and IL-1 beta by monocytes.	[19]
	Both preparations decreased the release of elastase induced by interleukin-8 (IL-8), neutrophil-activating protein-2 and formylmethionyl-leucyl-phenylalanine in polymorphonuclear leukocytes	[10]
	Suppresses tumor necrosis factor mRNA and secretion by endotoxin-stimulated THP-1, a human monocytic cell line. The mechanism proposed was the decreased activation of the NF-κB transcription factor	[20]
ALVEOFACT®	Concentration-dependent suppression of immunoglobulin production and cell proliferation in lymphocyte stimulated with phytohemagglutinin	[21]
	Similar behavior to CUROSURF® and VENTICUTE®; however, chemotaxis against autologous eosinophils was not induced	[15]
SURFACEN®	Inhibits the production of tumor necrosis factor-alpha in human monocytes resting with LPS	[22]
	Bactericidal effects against Gram positive and Gram negative bacteria	[23]
EXOSURF®	Suppresses TNF mRNA expression and secretion by the endotoxin-stimulated THP-1 human monocytic cell line. The mechanism proposed was the reduction of activation of NF-κB transcription factor activation	[20]
SURFAXIN®	This study was comparative with SURVANTA®. Surfactant-treated groups secreted less IL-8 than saline-treated cells, whereas SURFAXIN®-treated cells secreted the lowest levels of IL-6. SURFAXIN®-treated cells did not expressed matrix metalloproteinase 7, while SURVANTA® attenuated it only after 72 h and it was also present in saline-treated cells. Histology showed less injury with SURFAXIN® relative to SURVANTA® and saline	[24]
VENTICUTE®	Similar behavior as CUROSURF®: modulated viability of eosinophils, increased number of apoptotic and necrotic cells and levels of eosinophil cationic protein. However, it did not induce chemotaxis against autologous eosinophils	[15]
	Comparative study with CUROSURF®. Both surfactants inhibited the LPS-induced increase in TNF-alpha expression. VENTICUTE® induced higher expression of IL-10 than CUROSURF® and had no effect on IL-8 expression. However, CUROSURF® increased IL-8 expression at higher concentrations	[16]

by *Forest*, in October 1999, as a lung surfactant for the treatment of RDS in premature infants. In 1998, INFASURF® was approved by the FDA for preventing RDS in premature infants. From *Ikaria Holdings, Inc*, it is available in USA indicated to RDS. On the other hand, *Samaritan Pharmaceuticals Inc*. used the USA FDA approved regulatory file in preparing marketing applications for INFASURF® with regulatory authorities in Turkey, Serbia, Bosnia, Albania, Egypt and Syria to gain the drug country marketing authorization. In January 22, 2007, it was announced that *Samaritan* has signed an exclusive license for the marketing and sales of the USA approved INFASURF®, a specialist medication used to treat and prevent RDS in premature infants. Under this agreement, *Samaritan* has obtained exclusive rights to sell INFASURF® in Turkey, Serbia, Bosnia, Macedonia, Albania, Egypt and Syria.

SURVANTA®

Available on the pharmaceutical market, it is commercialized by two companies: *Abbott India Limited*, indicated to RDS in premature infants, and *Abbott Laboratories*. In December 12, 1993, Abbott received approval for SURVANTA® from Health Canada, and, in July 1st, 1991, from the USA FDA for SURVANTA® (25 mg/mL) intratracheal suspension. The USA FDA have granted the Orphan Drug Designation for SURVANTA® on February 5, 1986.

SURFAXIN®

It is currently in the phase of development and preclinical evaluations by two companies: *Esteve* and *Discovery Laboratories Inc*. In March 13, 2009, *Discovery Laboratories, Inc*. announced that it will advance its ongoing Phase II trial of SURFAXIN® for children up to 2 years of age with Acute Respiratory Failure (ARF). On July 29, 2008, *Discovery Laboratories*, *Inc*. announced the publication in *Pediatric Research* of data from a preclinical research study. Data showed that the KL-4 surfactant reduced the inflammatory response and improved cell survival and function in a hyperoxic-induced lung injury in an *in vitro* cell-culture model. *Discovery Laboratories, Inc*., announced in January 5, 2009 the publication of results from its Phase II clinical trial of SURFAXIN® for the prevention and treatment of bronchopulmonary dysplasia in *Pediatrics*. The SURFAXIN® standard dose had a lower incidence of death or bronchopulmonary dysplasia, a higher survival rate through 36 weeks post-menstrual age and fewer days on mechanical ventilation. On January 10, 2011, *Discovery Laboratories*, *Inc*., provided an update regarding its efforts to file a Complete Response intended to gain FDA authorization for marketing SURFAXIN® at US to prevent RDS in premature infants. *Discovery Laboratories, Inc*., and *Esteve* are developing with the use of SURFAXIN® a treatment for the Meconium Aspiration Syndrome and ARDS in adults.

SURFACEN®

It has been available in Cuba since 1995, indicated in NRDS. A comparative trial with SURVANTA® showed similar patterns of oxygenation and ventilation response, with a tendency to a higher increase in initial oxygenation in the SURFACEN® group [25]. Recent results, not yet published of a surveillance study showed the same adverse effects that in previous observations [26]. Also, in an efficacy trial, it demonstrated to improve oxygenation in adult patients with ARDS; however mortality was not reduced.

There is no doubt about the effectiveness of the surfactant clinical preparations in the NRDS therapeutics. After these confirmations, studies on the effectiveness of the synthetic *vs.* natural surfactant, its side effects, days of stay in intensive care, etc., were initiated. Several clinical trials have made comparisons within multicenter studies, having as conclusion that the natural surfactant is more effective and has fewer side effects. Surfactant preparations, containing SP-B and SP-C, have shown better results in clinical trial than using the synthetic lipid based surfactant without surfactant proteins [27]. Moreover, although it has been found that exogenous or synthetic surfactant preparations improve the different respiratory variables such as oxygenation, not all types of natural or synthetic surfactant have the same effectiveness [28-30]. In this sense, surfactant preparations containing higher concentrations of SP-B and SP-C give better responses than those with lower concentrations.

The potential use of the exogenous surfactant beyond the neonatal period is currently under study. Not any study found adverse effects with the use of natural surfactant in the neonatal population. The effectiveness of the exogenous surfactant in acute respiratory failure in pediatrics has been suggested by controlled preliminary studies, which showed improvement in the respiratory variables, but not in mortality. Recently, the first clinical trial reporting a positive impact on survival has been published. These results are encouraging.

Instillation of exogenous surfactant into the lungs of pediatric patients with acute respiratory distress syndrome (ARDS) has resulted in improved survival [31]. Despite this, intratracheal surfactant has not proved to be beneficial for adult patients with ALI/ARDS. Two large, randomized and controlled studies have been performed in adults using two different synthetic surfactant preparations [32, 33]. Neither study showed an improvement in survival. On the other hand, several small clinical trials using natural surfactant (bovine or porcine) have shown reduced mortality [34-36]. Nowadays, treatment with surfactant is not used routinely for the management of ARDS. The use of exogenous surfactant therapies for the treatment of ARDS in adults is still under debate, and probably depends critically on the development of new clinical surfactants.

Ongoing developments

In parallel with the clinical trials, there are new molecules or drugs being used in clinical diseases. They can be combined with clinical lung preparations.

The choice of an ideal or suitable surfactant for respiratory diseases other than NRDS goes through a rigorous basic research on structure-function relationships of the surfactant's components. Research on lung surfactant topics open new options to improve the lung surfactant preparations to be used in other

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lung diseases. The major objective is to overcome the inhibition of lung surfactant present in all lung diseases (Figure 1).

To achieve this goal, the ways of researches can be grouped as follows (Figure 2). Briefly we comment and update the major strategies to overcome the inhibition of lung surfactant. Optimization of lipids and proteins comprise increasing the phospholipids concentration, since the new surfactant preparations (SURFAXIN® and VENTICUTE®) have highest levels of synthetic hydrophobic proteins compared to exogenous lung surfactant preparations. These new preparations have been possible due to research and development in molecular biology and chemical synthesis of the hydrophobic surfactant proteins [37, 38].

The addition of SP-A is essential. None of the clinical surfactant preparations has SP-A; this protein is removed during the process to obtain these preparations. The anti-inflammatory and antimicrobial characteristics of SP-A [39-41] make it an attractive potential therapeutic agent. Scientists have to solve the following problems: SP-A is a very large and complex molecule, having all the posttranscriptional events, and, therefore, is very complicated to obtain human recombinant SP-A. On the other hand, it is needed a more complete understanding of its mechanism of action in the lung. Recently, Awasthi *et al*. [42] updated the clinical trials of SP-A preparations in ClinicalTrials.gov, and no clinical trial using SP-A was still found.

Polymers

The use of polymers is a very important topic. Taeusch *et al*. [43] and Kobayashi *et al*. [44] were the pioneers in the use of polymers as additives to clinical pulmonary surfactant. The proposed polymers' mechanism of action is a polymer-induced depletion-attraction model. It means, in general terms, that they promote surfactant aggregations and adsorption; therefore, polymers can neutralize the surfactant inactivation due to many inhibitors substances. The first polymers tested were dextran and polyethylene glycol, and also hyaluronic acid has been widely used. Many *in vitro* and *in vivo* studies [45-48] have shown polymers to improve the surface activity of surfactant preparations overcoming surfactant inactivation. Investigations on this topic are aimed at answering different questions: Which polymers? What molecular weight? Ionic or non-ionic polymers? What type of interactions occurs between clinical surfactant and polymers? What concentration? However, the use of polymers in combination with clinical surfactant preparations is undoubtedly an option for the development of new formulations to be used in ARDS.

Use of protease and phospholipase inhibitors and antibiotics in pulmonary surfactant preparations

Pharmaceutical compositions containing some low weight drugs have been delivered by pulmonary administration, but not all low-molecular-weight drugs, however, can be efficaciously administered through the lung. The pulmonary surfactant brings innumerable advantages, as a carrier, due to its own properties of spreading and inherent therapeutic potential.
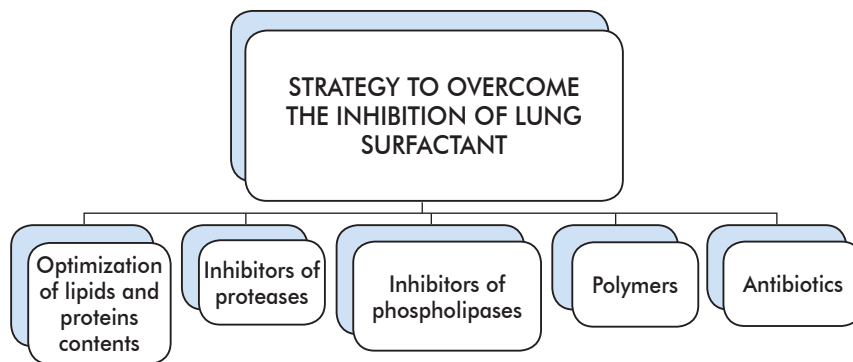

Figure 2. Strategy to overcome the inhibition of lung surfactant.

Therefore, it is expected that intratracheally instilled drugs are more effective when the distribution within the lung is optimized by using pulmonary surfactant as a carrier [49].

Inhibitors of proteases

Recently, in a review about human elastase inhibitor and lung surfactant in ARDS [50], we commented on proteases as obvious target for inhibitors-based therapies because they are involved in the physiopathology of the inflammatory pulmonary disease. Despite the numerous researches about the relationships between proteases, pulmonary surfactant and the pulmonary inflammatory disease, combinations of exogenous lung surfactant preparations with protease inhibitors are still insufficient. Nevertheless, this year *Discovery Laboratory* has launched a patent entitled 'Compositions for treatment and prevention of pulmonary conditions' [51]; the patent proves broad coverage compositions that employ a combination of certain pulmonary surfactants with a broad array of protease inhibitors for treating pulmonary inflammation. This patent, together with other reports, open a new possibility to use a mixture of proteases' inhibitors and pulmonary surfactant in the respiratory disease.

Phospholipase inhibitors

There are several studies confirming alterations of pulmonary surfactant by phospholipases in ARDS. Surfactant can be readily hydrolyzed by secreted phospholipases A_2 ($sPLA_2$), especially the type-IIA $sPLA_2$, and this process can contribute to the loss of surface tension-lowering properties of the surfactant [52]. The produced lysophospholipids and eicosanoids have been implicated in the pathogenesis of acute lung injury.

Specific inhibitors are available for $sPLA_2$ [53] and have also been used in clinical assays of acute lung injury/ARDS, being indole derivatives those used with the greatest success, among them LY315920Na/ S5920 [54] and LY311727. There are several investigations dealing with the phospholipase topic, although, up to date, there are no studies combining phospholipase inhibitors and the pulmonary surfactant (excepting the patent mentioned above in which phospolipase inhibitors, in addition to protease inhibitors, are also claimed). The use of surfactant preparation in combination with inhibitors of sPLA-IIA2

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can represent a promising strategy for the treatment of ARDS.

Another line of investigations is the obtainment of phospholipids resistant to hydrolysis by PLA$_2$; Notter's group has developed a novel diether phosphonolipid (DEPN-8) analog to DPPC [55]. Walther *et al.* [56] developed a fully synthetic lung surfactant constituted by plus DEPN-8 and Mini-B (synthetic analog to SP-B) designed by Waring *et al.* [57], which had a high surface activity and the ability to resist degradation by phospholipase, in inflammatory lung injury.

Antibiotics

Efficient antimicrobial therapy is considered to be dependent on appropriate antibiotic concentrations at the site of infection. Besides, the interaction between the pulmonary surfactant and the antimicrobial agent is very important to propose the use of an exogenous pulmonary surfactant as a drug delivery system for antibiotics to the alveolar compartment of the lung [49]. Especially, the combination of anti-microbial agents and surfactant offers an alternative for critically ill patients with pneumonia. Gram-positive bacteria like group B streptococci or *Staphylococcus aureus* and Gram-negative microorganisms, such as *Escherichia coli*, *Klebsiella pneumonia* or *Enterobacter cloacae*, are common pathogens causing serious neonatal infections, including neonatal pneumonia [58]. When searching Clinicaltrials.gov, no clinical trial combining pulmonary surfactant and antibiotics was found, but there were many studies evaluating different antibiotics in mixture with pulmonary surfactant with promissory results. For example, Polymyxin-B (PxB)-containing pulmonary surfactant is more resistant to inactivation by human meconium than modified natural surfactant *in vitro*; also antimicrobial activity of PxB against *E. coli* is maintained when PxB is added to pulmonary surfactant, and it is not significantly different from PxB alone [59]. More recently, this group showed that animals receiving pulmonary surfactant plus PxB had no difference in lung compliance compared with the pulmonary surfactant or PxB-treated group. Mixtures of PxB and pulmonary surfactant showed antimicrobial effects in neonatal rabbits and prevent systemic spreading of *E. coli* [58]. Four cationic additives, among them PxB, improved the surface activity of BLES® in the presence of serum [60].

Conclusions

The several scientific reports about immunomodulatory properties and clinical assays of the different surfactant preparations available today are consistent with the importance of pulmonary surfactant in the context of respiratory disease. In the near future, pharmacologic therapy based on the addition of different drugs (inhibitors of elastase and phospholipases, antibiotic or polymers) as ingredients of a surfactant, as well as optimizations of surfactant component preparations, could be effective in treating patients with severe respiratory disease.

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