Universidade de Lisboa

Faculdade de Farmácia

Síndroma do Desconforto Respiratório: surfactantes sintéticos na terapêutica de reposição do surfactante pulmonar

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Mestrado Integrado em Ciências Farmacêuticas

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Monografia de Mestrado Integrado em Ciências Farmacêuticas apresentada à Universidade de Lisboa através da Faculdade de Farmácia

Orientador: Doutora Lídia Pinheiro, Professora auxiliar

Co-Orientador: Doutora Célia Faustino, Professora auxiliar

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Abstract

The pulmonary surfactant is constituted by mainly phospholipids and 4 different surfactant proteins. SP-A and SP-D are the hydrophilic surfactant proteins which have a main role of defense. SP-B and SP-C are the hydrophobic surfactant protein which have properties that allow the surfactant to have reduce surface tension. This reduce surfactant tension in the main role of the pulmonary surfactant. The low surface tension in the air-liquid pulmonary interface on the alveoli facilitates gas exchanges and prevents the alveoli from collapsing. Also, one of the main phospholipids in the composition of surfactant is Dipalmitoylphosphatidylcholine (DPPC). Natural surfactant also has some cholesterol which modulates the fluidity of the surfactant membrane.

Acute Respiratory Distress Syndrome (ARDS) is a multifactorial disease which affect the pulmonary track and has a high mortality. ARDS can further be subdivided in Respiratory Distress Syndrome (RDS) and ARDS itself. RDS is a pathology which affects newborn babies in concrete, preterm infants. In this case, babies don't produce enough pulmonary surfactant because of the immaturity of the lung epithelia, in particular of type II pneumocytes which are the ones responsible for surfactant production and recycling. RDS can be effectively treated with exogenous surfactant.

On the other hand, ARDS does not respond so well to surfactant treatment, has the pulmonary edema characteristic of the pathology can inactive natural and exogenous surfactant. Also, there isn't a unified consensus on the dosage. In the case of newborns 100mg/kg or 200mg/kg of bodyweight seems to be the optimal doses, but when extrapolated to full grown adults that would be a huge amount of fluid to instill in the lungs. This makes the treatment more expensive to apply has well to investigate.

Another great technological innovation is the delivery of surfactants through aerosol with new aerosol generating technology. This is a much preferable way as it excludes the need for intubation and complications arising from that method. Although this is a fairly new method, its potential could represent a breakthrough in the way ARDS is treated.

Key Words: Pulmonary Surfactant, ARDS, Surfactant Proteins, Aerosol.

Resumo

O surfactante pulmonar é constituído principalmente por fosfolípidos e 4 proteínas do surfactante. A SP-A e a SP-D são proteínas hidrofílicas e têm como função principal a defesa do epitélio pulmonar. A SP-B e a SP-C são proteínas hidrofóbicas que possuem propriedades que permitem o surfactante ter tensões superficiais baixas. Estes baixos valores de tensão superficial na interface ar-líquido nos pulmões facilitam a troca de gases e previnem que os alvéolos colapsem. O dipalmitoilfosfatidilcolina (DPPC) é um dos fosfolípidos mais abundantes na composição do surfactante pulmonar. Este ainda possui também colesterol na sua composição que tem como finalidade modular a fluidez da membrana do surfactante.

O síndrome de dificuldade respiratória (ARDS) é uma doença multifatorial que afeta o aparelho respiratório e que se encontra associada a uma elevada taxa de mortalidade. O ARDS pode ainda ser subdividido em síndrome de dificuldade respiratória neonatal (RDS) e síndrome de dificuldade respiratória em si mesmo. RDS é uma patologia que afeta recém-nascidos em particular prematuros. Neste caso os bebés não produzem surfactante pulmonar suficiente devido à imaturidade do epitélio pulmonar, em particular dos pneumócitos tipo II, células responsáveis pela produção e recaptação de surfactante. Este tipo de síndrome de dificuldade respiratória consegue ser tratado eficazmente com a utilização de surfactante pulmonar.

Pelo contrário, o ARDS não responde tão bem ao tratamento com surfactante, uma vez que o edema pulmonar, que é característico deste tipo de patologia, pode inativar tanto o surfactante natural dos pulmões como um surfactante exógeno. Nesta patologia também não existe um consenso sobre que dosagem utilizar. No caso dos recém-nascidos 100mg/kg ou 200mg/kg de peso corporal parecem ser as doses ideais, mas quando estas são extrapoladas para um adulto, a quantidade de surfactante pulmonar seria demasiado elevada, encarecendo tanto o tratamento como a investigação.

Outro grande avanço tecnológico consiste na distribuição de surfactante pulmonar na forma de aerossol a partir dos novos métodos de nebulização. Esta é uma via preferencial uma vez que exclui a necessidade de intubação e as complicações associadas. Apesar de ainda ser um método relativamente recente, ele poderá constituir uma importante descoberta no tratamento do ARDS.

Palavras chave: Surfactante pulmonar, ARDS, Proteínas Surfactantes, Aerossol.

List of abbreviations

AECC - American-European Consensus Conference

ALI - Acute Lung Injury

ARDS - Acute Respiratory Distress Syndrome

BPD - Bronchopulmonary Dysplasia

CAG - Capillary Aerosol Generating

CPAP - Continuous Positive Air Way Pressure

DPPC - Dipalmitoylphosphatidylcholine

K - Lysine

L – Leucine

LB - Lamellar Bodies (LB)

SP - Surfactant Proteins (A, D, B, C)

PC - Phosphatidylcholines

PE - Phosphatidylethanolamine

PG - Phosphatidylgycerol

PI - Phosphatidylinositol

RDS - Respiratory Distress Syndrome

SM - Sphingomyelin

SRT - Surfactant Replacement Therapy

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Introduction

This work will approach the use of exogenous surfactant in the treatment of Acute Respiratory Distress Syndrome. This is a multifactorial pathology with a high mortality rate. One of the options of treatment is to use exogenous surfactant to replace the natural surfactant which is lowered in this disease. The actual challenge is to know how effective surfactant is in the treatment because of inactivation from endogenous proteins and inflammatory markers present, which of the different surfactants are better to use and how effective are the new technologies of delivering surfactant.

Objectives

With this work it will be tried to evaluate the effectiveness of the different types of surfactant has well has their effectiveness for the treatment of ARDS. It will also be assed the new delivery routes of surfactant to the pulmonary tract.

Materials

As a general rule of search, it was tried to use articles with less than 5 years, even though in some cases there wasn't information that recent so older journals where consulted. The main search engines used was google scholar and pubmed for articles and reviews, for clinical trials it was used the Cochrane library and ClinicalTrial.gov. It was excluded data published on journals who had low scientific credibility.

1.Pulmonary Surfactant Composition and Properties

Lungs must cope with surface tension and the interface between the hypophase fluid and the air. To do so they produce pulmonary surfactant which is a membrane-based system formed by lipids and proteins. It is secreted by type II pneumocytes into a thin layer that coats the respiratory surface. The surfactant fulfills two functions, it plays a biophysical role of preventing the alveoli's from collapsing by stabilizing the air exposed surface and reducing its tension, and a defense role. The absence of surfactant gives birth to a range of different pathologies, in which is included the Acute Respiratory Distress Syndrome (ARDS).(1) This syndrome will be covered in the next section.

Surfactant is mainly formed by amphipathic molecules and reduces the surface tension from water which is approximately 70mN/m in pure water at physiological temperature to 0mN/m preventing the collapse of the lungs.(2) This is achieved by polar groups in the surfactant establishing polar interactions with the surface water molecules and reducing the intermolecular cohesive forces. This material is composed not only by a monolayer of amphipathic molecules but also by a network of interconnected membranes between the interfacial film and the surface associated structures that act as a reservoir of surface active molecules. The surfactant also has a bilayer in the hypophase which is strongly connected to the monolayer (Figure 1).

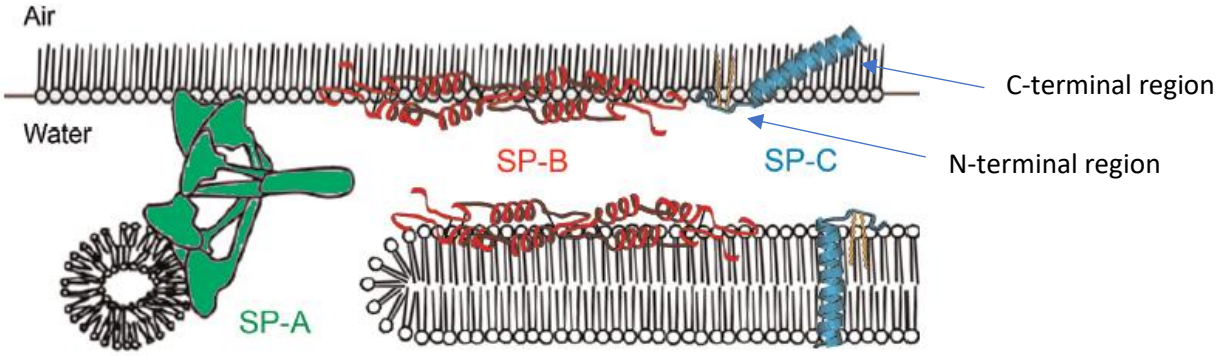


Figure 1. Membrane-associated pulmonary surfactant proteins. Schematic representation of surfactant proteins and their interaction with the monolayer and bilayer (from ref. 2)

It is synthesized in the endoplasmic reticulum and Golgi where the lipids and some of the surfactant proteins are combined, then multivesicular bodies are formed which mature into lamellar bodies (LB). LB will store and secret the surfactant(2). When it's release is need LB fuse with the alveolar membrane and occurs the exocytosis of the surfactant. This event can be triggered by different physiologic and pharmacological stimuli which will be addressed latter.(3)-(4)

1.1 Lipids

The surfactant mass is composed by 80% phospholipids, including zwitterionic phosphatidylcholines and anionic phospholipids, 5-10% neutral lipids, mainly cholesterol, and 8-10% proteins.

Phospholipids are amphipathic molecules with a polar and hydrophilic moiety and a hydrophobic side chain. The phospholipids are responsible for the active surface film in the liquid-air interface, they also form the matrix where the different surfactant structures are assembled. Phospholipids form bilayers in the type II pneumocytes, which is how the surfactant is stored. In the other hand in the surfactant film they form a monolayer with the headgroup towards the aqueous phase and the hydrophobic acyl chains towards the air. The higher the number of phospholipids in contact with air, the lower the number of water molecules in contact with it, which gives arise to lower surface tension. Therefore the energy needed for the lungs to expand, during the inspiration, is lower.(5)-(6)The heterogeneous organization of lipids, in both the monolayer and bilayer, with different melting temperatures and the influence of cholesterol (mention bellow in this section) give arise to properties such has compressibility, bending rigidity and permeability, and can alter the distribution and organization of membranes proteins.(2)

The main phospholipid is dipalmitoylphosphatidycholine (DPPC), representing 40% of the surfactant mass. DPPC is essential to produce low surface tension during compression because its' saturated acyl chains can adopt a highly lateral packed state. Surfactant also contains other phosphatidylcholines (PC) such as palmitoylmirystoyl-PC and unsaturaded PCs, such has palmitoyloleoyl-PC or palmitoylpalmitoleoyl-PC. Other functional important phospholipids are phosphatidylgycerol (PG) and Phosphatidylinositol (PI). These last two are hydroxylated anionic phospholipids and are thought to participate in selective interactions with the cationic hydrophobic surfactant proteins, which we will talk more about in the next section. At last there are also other phospholipids such as phosphatidylethanolamine (PE) and sphingomyelin (SM) which appear as minor components of surfactant. The most likely hypothesis is that this two come from other cell membranes. In addition, little amounts of lysophospahtidylcholine can be found.(6)

For the neutral lipids, the mains constituent is cholesterol. It is thought that cholesterol modulates the structure of the surfactant membrane by decreasing the packing of the phospholipids and improving the mobility. This fact is ought to cholesterol preventing phospholipids from suffering many isomerizations has they would if they were tightly packed without any cholesterol, giving some order to the surfactant, and making it more fluid.(5)

1.2 Proteins

The pulmonary surfactant has 4 different proteins which are Surfactant Protein-A(SP-A), Surfactant Protein-B(SP-B), Surfactant Protein-C (SP-C) and Surfactant

Protein-D(SP-D). While SP-B and SP-C are hydrophobic, SP-A and SP-D are hydrophilic.

SP-A, SP-B and SP-C are apolipoproteins because they are associated with phospholipids. SP-D can interact with phospholipids under specific conditions. It has been reported interactions between SP-D and glycolipids and fatty acids.(6) SP-A and SP-D are usually associated with host immune defense and SP-B and SP-C with surface activity of the surfactant.

SP-B is a saposine- like family protein which is highly hydrophobic. It has 79 polypeptide resides and 4 or 5 amphipathic α-helices connected by highly apolar loops. The helices have a high proportion of hydrophobic amino acids. SP-B has seven cysteines, six of them in strictly conserver positions where they form 3 disulphide bond, and the last forms an intermolecular disulphide bond giving arise to covalent homodimers. It has a net positive charge that enhances the interactions with anionic phospholipids. The protein is oriented parallel to the membrane surface establishing hydrophobic interaction between the membrane surface which seem to promote the interconnection of the membrane. This protein induces an increased permeability and aggregation of the phospholipid membrane that is essential for the surface activity of the surfactant.(6) This is due to the ability of SP-B to enhance interfacial absorption of phospholipids facilitating the refinement of the interfacial film during comprehension and consequent re-extension during expansion.(6)It allows the lipid transfer between the bilayer and monolayer. This protein seems to be the most important of the surfactant, since in studies the knockout of this protein in mice leads to respiratory failure while SP-C in the other and does not.(3,7)

SP-C is a small hydrophobic peptide with primary α-helical secondary structure. The peptide C-terminal region is enriched in branched aliphatic residues forming a highly hydrophobic α-helix. The N-terminal region has a positive net charge and has no defined secondary structure with 2 palmiorated cysteines. These cysteines help anchor the protein to the membrane has it adopts a transmembrane orientation with a 70º tilt.(2,6) This protein is essential to reach and maintain low surface tension on the film during high compression states and facilitates lipid exchange between the layers at this state(2).SP-C also stabilizes membrane-membrane and membrane-interface contact and has an apparent protective role for surfactant in the presence of cholesterol. There could be an indirect interaction between this two molecules, although it is still not proven.(8,9)SP-C is not essential to respiratory mechanism but it absence creates chronic and severe lung pathologies.(2)

The remaining proteins are SP-A and SP-D, has previously mentioned take part in the immune defense system, and can also be found in other epithelia(1). These are proteins from the colletin family constituted by mainly collagen and globular domains that modulate the inflammatory response, while also remove pathogens from the epithelial surface. They can recognize and opsone microorganisms and present them to the immune cells. SP-A binds to lipopolysaccharidespreferential from gram negative bacteria, while SP-D bind to peptidoglycans and lipoteichoic acid. This is achieved by the globular domain in both proteins that can bind to pathogens surfaces. This domain in the case of SP-A also binds to the surfactant membrane. SP-A also participates in the recycling and clearance of type II cells and macrophages. This is ought to SP-A binding

to DPPC. This interaction also might be also crucial for the formation of tubular myelin.(6) This protein also seems to promote vesicle aggregation when in the presence of calcium and enhance adsorption of surfactant into the interface. SP-D is required for the biogenesis of surfactant and its' packing into lamellar bodies by helping in the transfer of surface active phospholipids from the membrane to the air liquid surface.(10)

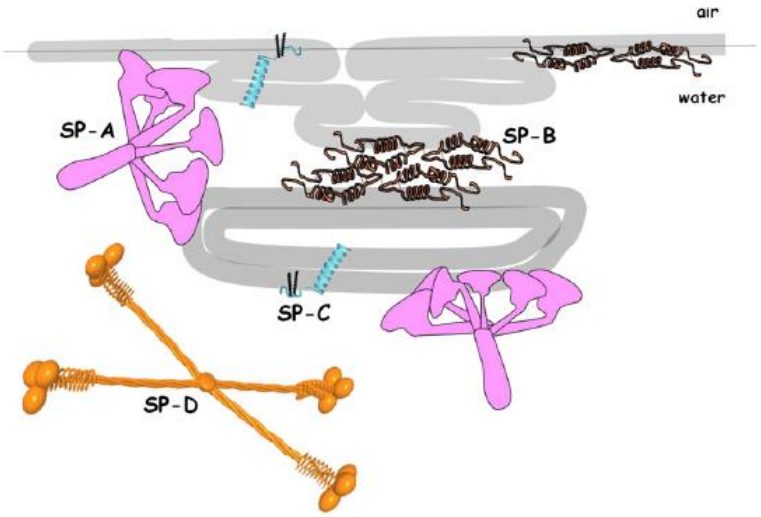


Figure 2. Structural models of surfactant proteins and their interaction with surfactant phospholipid layer (from ref. 5*), (grey bands represent monolayer/leaflets)*

2.Exogenous surfactant

Exogenous surfactants are a complex mixture of lipids and specific proteins in which the resemblance to the natural surfactant varies. There are three types of surfactants:

- Organic solvent extracts of lavage lung surfactant from animals (bovactant, bovine lung extract surfactant, calfactant).
- Organic solvent extracts of processed animal lung tissue. This can have or not synthetic components (poractant alfa, beractant).
- Synthetic preparations without material from animal lungs.

The first two mentions are the ones which have the closest composition and analogy to natural surfactant. The extract of lavage lung surfactant has all the natural surfactant phospholipids and proteins, although a great amount SP-A and SP-D is removed during lavage with organic solvents. The same happens to the surfactant from processed animal lung tissue, whose composition is similar. These types of surfactant have an additional issue which is they might contain cellular lipids and/or fragments of cellular proteins as well has prions. SP-B and other proteins can also be affected during processing of the surfactant, lowering their concentration.

The synthetic surfactants have the advantages over animal derived surfactant of being reproduceable, pure and having a greater manufacturing quality control efficiency. They

also are free from prion transmission and any culture or religious issue. These types of surfactant are challenging to bioengineer in the present time.(11–13)

It is now known that hydrophobic, proteins SP-B and SP-C, are essential to a rapid adsorption and spreading of the surfactant. On the other hand, the absence of SP-A and SP-D make the exogenous surfactants less immunogenic. SP-B is key to have an efficient lipid transfer to the interface and a cohesive multilayer organization. This organization is responsible for low surface tension during compression. SP-B is more active then SP-C on the interaction with lipids. Surfactants with only SP-B and with SP-B and SP-C have similar dynamic behavior and adsorption to interface. Also supplementation with SP-B or synthetic SP-B peptides increase the activity of surfactants containing only SP-C in animal models.(14,15) Concentration also seems to me a crucial factor for lower surface tensions, as high concentrations of surfactant are possible more efficient.(16–18)

The animal derived surfactant seems to be good enough for the treatment of Respiratory Distress Syndrome (RDS) in neonates, but not Acute Respiratory Distress Syndrome (ARDS) in adults. This might be because in ARDS there is inflammation and inhibition of surfactant while in RDS there is not. Further along these two topics, RDS and ARDS, will be accessed in detail. Curosurf® is the most concentrated surfactant with Survanta® coming in second. These concentrations arise from the differences in production method and properties like viscosity and lipid/protein ratio in the suspension. The last can prevent the preparations above certain concentration.(1)

Table 1. Composition and features of natural and synthetic surfactants. Adapted from [1]. SP-Ba and SP-Ca are recombinant forms of the proteins. PL stands for Phospholipid. Protein data are in µg/µmol of PL

	Natural				Synthetic			
Generic name	Calfactant	Bovactant	Beractant	Poractant alfa	Colfosceril	Lusupultide	Lucinactant	CHF5633
Regist. Trademark	Infasurf®	Alveofact ®	Survanta®	Curosurf®(in farmed approved)	Exosurf®	Venticute®	Surfaxin®	-
Animal origin	Bovine (BAL)	Bovine (BAL)	Bovine (minced tissue)	Porcine (minced tissue)	-	-	-	-
PL concentration	35 mg/ml	45 mg/ml	25 mg/ml	80 mg/ml	13,5 mg/ml	50 mg/ml	30 mg/ml	80 mg/ml
SP-Ba	-	-	-	-	-	-	2,7	0,2
SP-B	5,4	1,7	0-1,3	2-3,7	-	-	-	-
SP-C	8,1	-	1-20	5-11,6	-	-	-	-
SP-Ca	-	-	-	-	-	2	-	1,5

Although animal surfactants are effective in RDS, the high cost and limited production due to animal availability are major problems. That lead to the development of synthetic surfactants.(19)

The first synthetic surfactant, Exosurf®, had in its composition only lipids. At the present time it is known that the absence of SP-B and SP-C compromises the activity of the surfactant. Exosurf® has stopped being used has it shown worse activity then animal surfactants. Having said that, it is difficult to produce recombinant SP-B and SP-C due the high hydrophobicity, disulphite cross-link in in SP-B and the posttranslational modifications in the case of SP-C. Currently it isn't possible to get recombinant nature form of mature SP-B by overexpression in heterogenous system. That is because SP-B tends to be toxic to the cell of production. In the new generations of surfactants, it is being used proportions of saturated and unsaturated phospholipids and recombinant peptides that mimic SP-B and SP-C. These peptides incorporate functionally crucial regions of the proteins.

Some examples of this are KL4 (Lucinactant) which is a very simple peptide that mimics the SP-B behavior by showing a similar hydrophobic/hydrophilic pattern, and Super Mini-B. The N and C-terminal regions of SP-B are essential for is surface properties, Super Mini-B is a peptide that mimics the structure of this terminal regions. KL4 peptide, also called Sinapultide, as repeated unites of both leucine(L) and lysine(K). Lucinactant also has greater resistance to oxidation and inhibition proteins. Both KL4 and Super Mini-B show improved oxygenation in studies.(1) Also there have been novel lipids design to have more beneficial molecular properties such has phospholipase resistance. DEPN-8 is one of those lipids showing phospholipase resistance, which can be very important has ARDS inflammation mediators can break phospholipids.(11) CHF5633 is a newly designed synthetic surfactant which contains both hydrophobic proteins, a variant of 33 aminoacids of SP-C and Mini-B peptide. It has been shown in animal studies that it improves lung function as well has lung compliance. It also has high phosphatidylglycerol content which seem to prevent the lung inflammation from ARDS in lambs.(20)Venticute®, a recombinant C protein surfactant, has shown improved oxygenation in patients with ARDS, but not improved mortality with when the causes of ARDS are heterogeneous. However when we are present with ARDS from pneumonia or aspiration of gastric, which has a severe oxygenation deficit, there might be a survivability benefits.(21)

2.1 Comparison between different surfactants

Table 2. Comparison between animal and synthetic surfactants, adapted from [19]. RCT-Random Clinical Trial, NICU - Neonatal intensive care unit

Study	Surfactant Preparations	Study Design	Results
Bloom et al., 1997(22)	Calfactant , beractant	Prospective, multicenter, double-blind, RCT, 31 NICU	No differences in the pneumothorax and mortality or survival without BPD, longer duration of treatment effect in calfactant than beractant
Soll and Blanco, 2001(23)	Colfosceril, beractant, calfactant, porfactantal alfa	Meta-analysis 11 RCT	Higher mortality and pneumothorax in the colfosceril compared to animal-derived surfactants
Ramanathan et al., 2004(24)	Poractant alfa, beractant	Prospective, multicenter, masked, RCT	Less mortality, redosing of surfactant, and oxygen supplement in the 200mg/kg of poractant alfa
Ramanathan 2009(25)	Beractant, calfactant, poractant alfa	Meta-analysis 14+8 RCT, 20 000 infants	No differences between beractant and calfactant, benefits in weaning of ventilator, redosing and survival in high-dose of poractant alfa
Singh et al., 2011(26)	Poractant alfa, beractant, calfactant	Meta-analysis, 5 RCT, 529 infants	Reductions in deaths and the need for redosing with high-dose poractant alfa but not low-dose poractant alfa
Trembath et al. 2013(27)	Beractant, calfactant, poractant alfa	Multicenter, RCT, 332 NICU, 51 282 infants	Similar effectiveness in prevention of air leak syndromes, death and BDP or death
Singh et al., 2015(28)	Beractant, calfactant, poractant alfa	Meta-analysis, 16 RCT	No diferences in death or chronic lung disease in calfactant, beractant or porfactant alfa.
Moya etal., 2005(29)	Lucinactant, colfosceril, beractant	Multicenter, double-blind, RCT, 1 294 infants	Reduction in the incidence of BPD in lucinactant compared with colfosceril, reduction in the RDS-related mortality in lucinactant compared with beractant.
Sinha et al., 2005(30)	Lucinactant, poractant alfa	Multicenter, RCT, 253 infants	Similar in efficacy and safety.
Ardell et al., 2015(31)	Colfosceril, beractant, calfactant, porfactant alfa	Meta-analysis 15 RCT	Reduction in the risk of pneumothorax and mortality in animal derived surfactant rather than colfosceril
Chiesi Farmaceutici S.p.a.(32)	CHF5633, Porfactant alfa	Multicenter, double blind, RCT, 123 infants	Recently completed, no results available yet

With the increased interest in using synthetic surfactants has mentioned above, it is important to evaluate and compare the efficacy between animal and synthetic surfactants. As shown in table 2 there are no significant differences in the animal surfactants as they all show similar outcomes independent of which one is used, with porfactant alfa being slightly superior. On the other hand, Lucinactant seems to be superior to some animal surfactant and similar to porfactant alfa. As for Venticute® and CHF5633 there aren't any comparison clinical trial at the moment of this review. Despite this there is an *in vitro* and *in vivo* study on the second generation surfactant, CHF5633, which has shown this surfactant to be as effective porfactant alfa to treat ARDS.(33)

3. Acute Respiratory Distress Syndrome

The American-European Consensus Conference (AECC) in 1994 defined acute lung injury(ALI) as a respiratory failure of acute onset with a PaO_2/FiO_2 ratio $\leq$ 300 mmHg and Acute Respiratory Distress Syndrome (ARDS) as a PaO_2/FiO_2 ratio $\leq$ 200 mmHg(34). In 2012 the Berlin definition of ARDS was described by timing, radiographic changes, severity and origin of edema. It could be classified has mild, moderate or severe according to the PaO_2/FiO_2 ratio.(35)

3.1 Respiratory Distress Syndrome

Respiratory Distress Syndrome (RDS) is one of the most common reasons for hospitalization of infants in critical care. Premature birth and cesarean delivery increase the risk of developing RDS. Surfactant deficiency, Intrauterine growth restrictions and lung immaturity are the main causes of this pathology. Its clinical manifestations are tachypnea, nasal flaring, grunting, intercostal or subcostal retractions and cyanosis. The newborn can also exhibit lethargy, poor feeding, hypothermia and hypoglycemia. The previously mention all manifest immediately after birth and worsen in the first 12 to 14 hours.(36–39)

In the present the debate about the best treatment option for RDS is still open. There are some different approaches like using exogenous pulmonary surfactant, Continuous Positive Air Way Pressure (CPAP) and antenatal corticosteroids. The last mentioned has been shown to reduce the severity of RDS and decrease the mortality, but not the incidence.(36,40)

Surfactant Replacement Therapy (SRT) has shown to decrease the mortality, pneumothorax, pulmonary interstitial emphysema and survivability without bronchopulmonary dysplasia (BPD). There also is evidence of the additive benefits of using antenatal corticosteroids and SRT, which have a greater improvement on lung function when combined than used alone.(13,40) Still there is a lot of uncertainty on Surfactant Replacement Therapy, which type of surfactants to use, mode of administration (which will be addressed latter) and timing of the surfactant. Regarding the timing, it has been studied the use of prophylactic surfactant, which is used before the onset of RDS in neonates, who fit the criteria for developing it, and rescue surfactant, used after the development of the pathology, usually within a 12 hour window after

birth.(40) The prophylactic surfactant approaches is being abandoned because it was seen an increase in chronic lung disease associated with mechanical ventilation, even though there was an reducing on mortality.(41) As for the rescue surfactant, it can be subdivided in early rescue, 1-2h after developing RDS, or latte rescue, more than 2 hours. It seems that the early rescue is the most effective way to improve the pathology and decrease death and complications. The late rescue surfactant shows worse results then the prophylactic use.(40) It's still unclear which is superior, rescue or prophylactic surfactant on decreasing the risk of BPD. Having said that, SRT comes with the disadvantage that the patience has to be intubated for administration, and in the case of neonates, this process can easily harm the lungs.(41,42)

The CPAP technique also has shown to be an effective approach. Newborns with risk of developing RDS are immediately and this has shown to decrease the need for SRT and preventing in some cases the pathology. Also, CPAP, especial nasal continuous positive air pressure, which is a less invasive method, has increased has a first line treatment in RDS. CPAP has shown decrease in mortality and in the risk of developing BPD.(13,40,42)However in infants who CPAP isn't enough to treat the disease, they lose the benefits of early surfactant administration, as they are intubated later.

Another recent technique is INSURE. INSURE consist in intubating, administering surfactant, then extubating and using CPAP. This technique looked promising, but in recent studies there wasn't shown any benefit over CPAP. This is because even short intubation can lead to lung damage, and that it is difficult to extubate neonates, especially preterm newborns. INSURE has not shown any benefits in higher survival without BPD when compared to the other options.(42)

It is also worth mentioning that the use of budesonide together with surfactant have had promising result in improving mortality and incidence on BPD without showing any long term development side effects. Surfactant is a good vehicle for budesonide, making this seem a good option.(42)

3.2 Acute Respiratory Distress Syndrome

In adults and children, in contrast with neonates there is presence of extensive inflammatory exudates in the lung from damage and leak across the alveolar-capillary barrier. There are different causes of ARDS in this age group. The direct lung causes like pulmonary viral or bacterial infections, gastric aspiration, near drowning, thoracic radiation, blunt thoracic trauma with lung contusion and inhalation of smoke or other toxicants. In the other and there are the indirect/extra-pulmonary causes like sepsis, hypovolemic shock, generalized trauma with long bone fracture, multiple transfusions and pancreatitis. In the systemic causes surfactant replacement therapy doesn't seem to have any benefits on mortality, therefor we will mainly address SRT in direct lung causes.(3,12)

As a result of edema and inflammation there is a detriment of physicochemical interactions between substances in the alveoli leading to the impairment of the surfactant. This can be done by different inhibitors mainly, plasma, blood proteins like albumin, cell

membrane lipids, fluid free fatty acids, reactive oxidants and lytic enzymes like phospholipases and proteases. Albumin and blood proteins lower the surface activity due to competitive adsorption to the air liquid interface, reducing the entry of surface active components into it. Cell membrane lipids, lysophospholipids and fatty acids can mix into the surface film and compromise its ability to lower surface tension. Lytic enzymes and reactive species can alter the function of surfactant lipids and proteins.(12)

There are also other specific mechanisms of surfactant disfunction. Alterations in alveolar surfactant aggregates where the highly active large surfactant aggregates, being composed by lamellar bodies, tubular myelin and large multilamellar vesicles, are reduced in activity and/or content, and the less active small aggregate, composed by unilamellar vesicles, become more prevalent lead to surfactant disfunction. Also altered synthesis, secretion and composition of surfactant due to injury induces changes in type II pneumocyte is observed.(11,43)

It important to keep in mind that ARDS is a dynamic process and that the initial acute inflammation and surfactant disfunction can evolve to chronic or sub- chronic inflammation, fibroproliferative repair and vascular remodeling. The previous happen several days after the acute phase of the inflammation. In this period, it is observed proliferation of type II pneumocytes and dedifferentiation to type I pneumocytes. It is theorized that surfactant intervention is most effective on the early stages before this process happens. Before diving in the different approaches of surfactant usage it is worth mention that in adults, contrarily to neonates, positive airway pressure can sometimes result in lung injury within minutes.

3.3 Efficacy of surfactants in the treatment of ARDS

Although it is well established the efficacy of surfactant replacement therapy for RDS it is not for ARDS. That is because this pathology can have different causes, has mentioned above, and they can be from direct or indirect lung injury. Moya and colleagues(44,45) observed while studying surfactant treatment in children with Surfacen®, which is a porcine origin surfactant, that there was an improvement in oxygenation and mortality from ARDS. Even though these results are promising both of the trials had small clinical samples. Another trial from Markart and colleagues (46) showed improved gas exchanged and normalization of endogenous surfactant compositions after treatment with Venticute® in adults. On the other hand Spragg and colleagues(47) in 2 multicenter, double blind and randomized control trials with venticute® shown that there was no improvement in mortality although there was improvement in gas exchanges in adults. At last Lu and colleagues(48) and Douglas and colleagues(49) both shown that there was no benefit of using exogenous surfactant in ARDS. Both studies used animal derived surfactants. Lu's study demonstrated that surfactant replacement therapy reaerates poorly non aerated lung areas but also increased lung tissue in normalized areas there for not improving gas exchanges. Wilson's trial has shown no improvement on oxygenation nor mortality. From all the relevant trials on this subject there is also Kesecioglu and colleagues(50) which has shown increased in mortality with an animal derived surfactant. This result might be due to the surfactant

formulation. In this trial surfactant was obtain in a 100 ml vial in powder, to then be dispersed with 60ml of saline. This might have compromised the proteins and phospholipids structures making the surfactant inactive.

From these different trials it is possible to draw some conclusions. First surfactant replacement therapy for ARDS seems to be more effective in children than in adults. This might be due to alveolar-interdependency and alveolar compliance but also because children usually have less severe lung injury, and better recovering then adult patients.

Alveoli are heterogeneous in size and geometry. These inter-alveolar differences have an impact on their mechanical behavior. Alveolar surface tension varies through thickness of surfactant layer. After exogenous surfactant administration there is an increase in size of small alveoli either because greater surfactant distribution or increase of the surface layer due to the smaller alveolar surface when compared to bigger alveoli. Alveolar compliance is directly proportional to alveolar dimension, so smaller alveoli have lower mechanical compliance and benefit more from surfactant distribution as indicated by the increased surface area. The distending pressure is equal to surface tension plus the elastic distending pressure. Therefore, by lowering the surface tension in terminal airways there is a decrease in distending pressure for all alveoli regardless of the size and compliance. Also, surfactant seems to favor most alveoli smaller than 20,000 μm^2. This might explain why surfactant therapy is more effective in children.(51)

From the above mentioned trials, it can also be extrapolated that there isn't still a definitive answer if surfactant therapy is beneficial in adults. It seems that the bad results can be because even after administrated, exogenous surfactant can be easily inhibited by inflammation factors and proteins from edema present in the lung. Also, it seems that distribution of surfactant is a key factor on its efficacy has seen in Lu and colleagues' trials where it reaerates the poorly aerated areas in the lung but increases lung tissue in normalized areas. In all the studies mentioned the surfactant was administrated through intratracheal intubation. New administrating techniques are being developed and surfactant resistant to inhibition, and even though these are still new areas and there isn't still a lot of data to compare it to, they will be addressed in the next section.

3.4 Surfactant dosing

At the present time of this monography, there isn't still a consensus in which are the optimal doses for the treatment of ARDS. In the case of RDS 100 mg/kg of body weight is the standard of care. Although 100 mg/kg is an excess comparing to the surfactant amount necessary to cover the whole alveolar network with surfactant film, about 3mg/kg, the excess can provide a reservoir of material in the hypophase that can adsorb into the air-liquid interface when needed. It also can incorporate endogenous surfactant via recycling by the type II pneumocytes.(1,12)

In ARDS he doses of 100mg/kg of body weight can correspond to 90-280ml of fluid volume. It is important to optimize the therapy doses and minimize the volume instilled because of edema and respiratory failure, nevertheless increased surfactant volume can impact distribution in the lung which is already compromised by the

previously mentioned. It has been verified in trial that higher surfactant volumes improved distribution.(12)

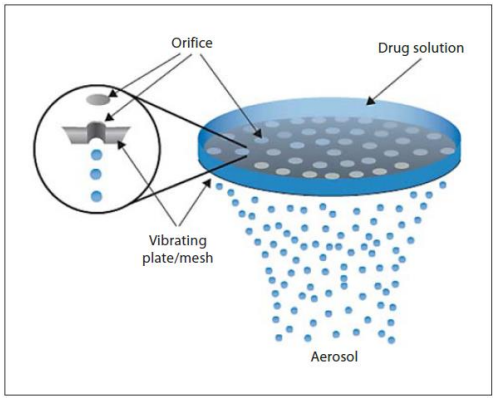
Even though there aren't any standard doses, in trial evaluating efficacy of surfactant in ARDS, doses of 200mg/kg at the time at 0, 12 and 36 hours, to a total of 600mg/kg could improve gas exchanges(48). Trials using low doses has 50mg/kg didn't shown much improvement in oxygenation or mortality.(49) Moya and colleagues trial(44) with 9 separate doses in an interval of 8hours each to a total of 100mg/kg seem to have improvement in oxygenation and survivability in children, who seem to be the best responsive group, with ARDS, to surfactant therapy. Also, there was verified an improvement in gas exchange while using 50mg/kg of Venticute® in 3 separated doses (0, 48 and 120 hours). All the other trials except the last mentioned used animal derived surfactants.

From the date found we can extrapolate that in animal derived surfactants doses between 100mg/kg and 200mg/kg should be safe and present improvement in oxygenation, has well has multiple administrations of the surfactant instead of single administrations, until the maximum concentration of 600 mg/kg bodyweight. In synthetic surfactants as Venticute® all the data found indicates that the standard use is of 50mg/kg, and it shows good results.

3.5 New administration techniques

The normal administration technique, bolus endotracheal surfactant delivery, even though it's effective in RDS and shows some positive results in ARDS, it can have some complication has trachea obstruction, alterations in cerebral flow, hypotension, hypoxemia, mechanical damage.(1)To counter this complications, aerosol delivery of surfactant was theorized. It would have theoretical vantages like minimal manipulation of respiratory track, improved pulmonary distribution, avoidance of acute airway fluid load immediate after surfactant instillation. Also the gradual surfactant administration could reduce the side effects as transient airway obstruction and reflux, hypercapnia, hypoxia, and may contribute to a more stable systemic and cerebral hemodynamics.(52) The first studies with jet nebulizer and nebulization of surfactants had poor results, has these aerosols had poor alveolar deposition and the particles need to be smaller than 5µm to bypass the upper airway. This technology was abandoned for a lot of years, until recently when it appears two new technologies which were vibrating membrane (Paria E-flow) and Capillary Aerosol Generating (CAG). These 2 new methods of aerosol dispersion have shown increased effectiveness on surfactant delivery. The vibrating membrane nebulizer shows significant improvements in efficiency and effectiveness of aerosolization. Aerosol droplets are generated by a perforated vibrating membrane mesh which can be customized to fit particles physicochemical

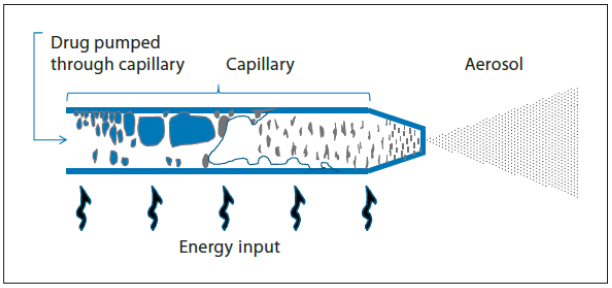
Figure 3. Vibrating mesh nebulizer, from[53]



properties like size.(53,54) As low stress is exerted on the fluid, aerosolization of fragile molecules without desaturation, like protein and genes, are possible.

Capillary aerosol generating is another promising technology as it is able to produce a low flow, high output rate and has a customizable particle size. The aerosol is generated via heated capillary where the liquid from the surfactant is sucked and dispersed uniformly. This device has not been yet described in the literature for treatment of either RDS or ARDS.(53)

Figure 4. Capillary aerosol generator, from [53]



With these new approaches KL4 was tested in newborn pigs and porfactant alfa in newborn lambs, both using vibrating membrane nebulizer, and it was found that in both cases surfactant reached the lung still active after aerosolization. In a 2015 Hutten and colleagues animal trial with porfactant alfa for treatment of RDS(55), it was shown that humidified air on nebulization improves the PaO_2 when compared to non-humidified air. In this case it was used 861mg/kg total of surfactant. Also, longer periods of nebulization where preferred has the 30minutes and 60 minutes nebulization showed worse results than the three hours one even though it was being nebulized more surfactant per minute. It was shown that the higher doses had the best improvements and low doses had almost no effect. Also, the shorter times might prevent the surfactant to reach the lower lung lobes. The nebulized surfactant reached the lower lung lobes, opposite to what happens with the instilled surfactant which usually only reaches the upper lung lobes. This might be from the usage of CPAP in concomitance with the surfactant. On the other, this might deposition contribute to a better effect of surfactant nebulization as it shows a more homogenous distribution. Pillow and colleagues(53) verified as well that enhanced homogeneity in distribution arise from the aerosolized surfactant when compared to the liquid instilled. Also, they found that using nebulized synthetic KL4 surfactant increased specific compliance, tidal volume and reduce anti-inflammatory markers.

Ricci and colleagues in 2019(52) used e-flow to study porfactant-alfa in animals with RDS. They used redispersed aerosol of undiluted porfactant alfa in doses of 100, 200, 400 and 600 mg/kg of bodyweight. In the doses from 200-400 mg/kg they obtain similar responses to bolus intratracheal use of 200mg/kg of surfactant with improved oxygenation. The doses of 100 mg/kg didn't show any benefits as it seems to be too low to produce effects. The doses of 600mg/kg also had a lower benefit than 200 and 400 mg/kg doses, this might be because there is surfactant accumulation in the airway.

There still isn't any data of the use of aerosolized surfactant in ARDS, but from the benefits presented in RDS, this might be a new way for treating the respiratory component of the disease. As aerosolization is a less invasive method, and both porfactant-alfa (which seems to be the better animal surfactant because it has higher concentration per milliliter) and both new synthetic surfactants like KL4 surfactant and Super Mini-B peptide surfactant (with their inhibition resistance properties) might be the way forward. Phase 2 clinical trials from Chiesi Farmaceutici S.p.a. with aerosolization

of surfactant in vibrating membrane nebulizers for the treatment of RDS are now on going and in the future might be used to treat ARDS has both pathologies are similar.

Conclusions

The development of new synthetic surfactants for the treatment of ARDS might be the key for future success in the therapy. There are still a few barriers that need to be overcome. Surfactant proteins B and C are now known to be the most important proteins of the surfactant and key to be able to achieve low surface tensions. Although is still very difficult to synthesis these proteins, in particular SP-B due to its complex structure and toxicity to the production cell, there are new analogue peptides that can mimic them with promising results. The presence of both this proteins in the surfactant seems to be essential to the effectiveness of it. CHF5633 is a new synthetic surfactant with both analogues of SP-B and SP-C and is now on Phase 2 clinical trials comparing it to Curosurf® which is the one who shows most efficacy as an animal derived pulmonary surfactant in the treatment of ARDS. Also, this CHF5633 is inhibition resistance, which can give arise to even better result in ARDS, where there are a lot of inflammatory mediators that inhibit natural surfactant.

Another promising idea is the aerosolization of surfactants that seems to enhance pulmonary distribution and is a way less invasive method when compared to intratracheal instillation. Combining aerosolization with CHF533 might be the way to go in the future for an effective treatment of ARDS. It is also important an investigation on the bioengineering of both surfactant proteins B and C to make better analogues and decrease the cost of production, making the methods of productions improve over time.

So, in conclusion there is still a lot of researched needed to make surfactant the standard treatment for ARDS but in time with the improvement of synthesis and delivery method of surfactants this might me the future.

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