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REVIEW

Alveolar Type II Cells and Pulmonary Surfactant in COVID-19 Era

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Summary

In this review, we discuss the role of pulmonary surfactant in the host defense against respiratory pathogens, including novel coronavirus SARS-CoV-2. In the lower respiratory system, the virus uses angiotensin-converting enzyme 2 (ACE2) receptor in conjunction with serine protease TMPRSS2, expressed by alveolar type II (ATII) cells as one of the SARS-CoV-2 target cells, to enter. ATII cells are the main source of surfactant. After their infection and the resulting damage, the consequences may be severe and may include injury to the alveolar-capillary barrier, lung edema, inflammation, ineffective gas exchange, impaired lung mechanics and reduced oxygenation, which resembles acute respiratory distress syndrome (ARDS) of other etiology. The aim of this review is to highlight the key role of ATII cells and reduced surfactant in the pathogenesis of the respiratory form of COVID-19 and to emphasize the rational basis for exogenous surfactant therapy in COVID-19 ARDS patients.

Key words

Pulmonary surfactant • Alveolar type II cell • SARS-CoV-2 • COVID-19 • ARDS

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Introduction

Pulmonary surfactant is a unique lipoprotein complex that lines the inner surface of the alveoli and small airways (Johansson and Curstedt 1997). From the first breath of the newborn, surfactant plays a key role in the physiology of the respiratory system. It reduces the surface tension at the air-liquid interface and prevents alveolar collapse, stabilizes the alveolar-capillary barrier, prevents edema, and allows for effective gas exchange and oxygenation (Mirastschijski *et al.* 2020). In the most common pulmonary form of the disease caused by novel coronavirus, SARS-CoV-2 enters and causes injury to target cells including type II alveolar (ATII) cells that express angiotensin-converting enzyme 2 (ACE2) (Bezara *et al.* 2020). Disruption of surfactant metabolism has wide consequences arising from its multiple functions. During the COVID-19 pandemic, surfactant attracted attention especially because of the defensive and immunomodulatory properties of surfactant lipids and proteins (Han and Mallampalli 2015). Surfactant dysfunction or deficiency in COVID-19 has typical pathophysiological, morphological, and clinical manifestations (Ochs *et al.* 2021, Gattinoni *et al*. 2021). The treatment for respiratory failure in acute respiratory distress syndrome (ARDS) is based on the current understanding of the central role that ATII cells and surfactant play in SARS-COV-2 infection and is inspired by the success of the use of surfactant replacement therapy in neonatal RDS (Koumbourlis and Motoyama 2020).

Pulmonary surfactant and its role in the host immune system

The respiratory system is a large interface between the body and the external environment. It is permanently exposed to toxins and pathogens and therefore requires effective local defense (Kolomaznik *et al.* 2017). Pulmonary surfactant is a complex substance that is synthesized and secreted by ATII cells. It contains about 90 % lipids and 10 % proteins, including the specific proteins SP-A, SP-B, SP-C, and SP-D (Johansson and Curstedt 1997). Surfactant covers the inner surface of the lungs and reduces surface tension at the alveolar surface to prevent collapse and facilitate gas exchange. It also prevents lung edema, relaxes the airway smooth muscle, and possesses multiple immune functions (Han and Mallampalli 2015). Surfactant, together with epithelial and endothelial cells, is part of the alveolar-capillary barrier. This barrier is susceptible to bacterial and viral infection that initiates a number of pathological events and finally leads to barrier breakdown (Gotts *et al.* 2014, Nova *et al.* 2019). Its dysfunction is followed by influx of neutrophils, protein leakage to alveolar space, edema, cell apoptosis and necrosis, and the inhibition of pulmonary surfactant, which are all typical signs of acute lung injury.

SP-A and SP-D are pulmonary collectins. They act as opsonins and bind to viruses to facilitate pathogen removal and regulate the function of inflammatory cells (Wright 2005, Watson *et al.* 2021). Disruption of this function leads to higher susceptibility to viral and bacterial infections. SP-A and SP-D prevent infection of epithelial cells through viral neutralization, agglutination, and enhanced phagocytosis (Hartshorn 2010). Pulmonary collectins also bind to the glycoproteins of viruses, including HIV, RSV, and SARS coronavirus, and to the hemagglutinin and neuraminidase of influenza A virus to inhibit their activity (Han and Mallampalli 2015, Kishore *et al*. 2020). Recently, the role of the genetics of innate immune molecules, SP-A1 and SP-A2, and their differential impact on the local lung microenvironment and host defense was addressed as a background of diverse expression of COVID-19 (Tekos *et al*. 2020). A recombinant fragment of human SP-D (rfhSP-D), composed of homotrimeric neck and carbohydrate-recognition domain, was shown to interact with S1 protein of SARS-CoV-2 and its receptor-binding domain in a dose-dependent manner. sfhSP-D acts as an entry inhibitor of SARS-CoV-2 infection by restricting the viral entry into HEK293T cells overexpressing angiotensin-converting enzyme 2 (ACE2) receptor. Treatment with 1.67 μM rfhSP-D inhibited viral replication in clinical samples of SARS-CoV-2-positive cases by ~5.5-fold and was more efficient than remdesivir (100 μM) in Vero cells. An approximately two-fold reduction in viral infectivity was also observed after treatment with 1.67 μM rfhSP-D (Hsieh *et al*. 2021, Madan *et al*. 2021).

SP-A and SP-D in the circulatory system may serve as novel markers that can be used in the clinical course, prognosis, and follow-up of patients with COVID-19 (Kerget *et al*. 2020, Ghati *et al.* 2021), in addition to IL-6 or KL-6 (Krebs von den Lungen) produced by damaged or regenerating ATII cells (d'Alessandro *et al*. 2020).

SP-B and SP-C possess distinct immunomodulatory properties besides their stabilizing effect on surfactant film (Mulugeta and Beers 2006, Glasser *et al.* 2009) and also as a part of synthetic surfactant preparations (Mikolka *et al.* 2021). The anti-inflammatory features were also demonstrated for protein-free lipid mixtures, DPPC (Gille *et al.* 2007), phosphatidylglycerol (Numata *et al*. 2010), and its analogs (Kandasamy *et al*. 2016) or some minor anionic surfactant phospholipids (Kuronuma *et al.* 2009, Voelker and Numata 2019). Surfactant lipids inhibited infection by the pandemic H1N1 influenza virus in several animal models (Numata *et al*. 2020).

Surfactant as a surface active agent, a "soap," may penetrate the virus coat, split it, and let the content be released into the environment. The new coronavirus may suppress the surfactant production in ATII cells to survive by using the ACE2 receptor in the infected lung tissue (Takano 2020). As a consequence of this hypothesis, pulmonary surfactant or its stimulants may be effective in the treatment and prophylaxis for COVID-19 (Takano 2020). Others also draw attention to the lipid pattern regulation in the respiratory system as a possible target of both treatment and prophylaxis against COVID-19 and other enveloped virus pneumonia (Mandato and Vajro 2021).

Alveolar type II cells as a target for SARS-CoV-2

The alveolar epithelium is made of alveolar type I (ATI) cells and ATII cells. ATII cells are small cuboid-shaped cells with a surface area of about 250 μm^2.

They represent about 5-7 % of the alveolar epithelial cells and have a typical morphology with lamellar bodies and microvilli at the apical surface (Mason 2006, Beers and Moodley 2017). The importance of alveolar type II cells has increased, as the lung is the major, although not exclusive, target organ in SARS-CoV-2 infection. ATII cells have multiple roles. They synthesize, secrete, and recycle pulmonary surfactant, which is an important factor for alveolar stability and host immunity (Lopez-Rodriguez and Perez-Gil 2014). ATII cells produce other substances that affect the function of the immune system, such as cytokines, growth factors (Zissel *et al.* 2000), and endogenous antimicrobial peptides (Hiemstra *et al*. 2016, Nova *et al*. 2020). They serve as progenitors of ATI cells and, as such, contribute to alveolar epithelial repair and regeneration (Ruaro *et al*. 2021). Distinct subpopulations of ATII cells play the progenitor role in a different context. ATII cell subgroups behaving as stem cells during steady-state replacement are probably different from cells engaged in lung repair after injury. Moreover, some of the ATII cells may differentiate into ATII to ATI transition subpopulations (Chen and Liu 2020).

Damage or depletion of this population can lead to various pulmonary pathologies, including those related to the pulmonary form of COVID-19. ATII cells stabilize the alveolar-capillary barrier and are involved in lung fluid homeostasis and the maintenance of fluid-free alveoli by participating in sodium transport through sodium channels (Mason 2006). In acute lung injury, inflammatory cells infiltrate the alveoli and overproduce TNF-α, which may affect epithelial sodium channels and cause pulmonary edema (Yamagata *et al.* 2009). Down-regulation of alveolar Na/K-ATPase is also associated with pulmonary edema, as was shown in experimental models of lung injury as well as in clinical settings (Sznajder *et al.* 2002).

Angiotensin-converting enzyme (ACE2) protein is found in some airway cells and is highest within regions of the sinonasal cavity and pulmonary alveoli, which are the sites of viral transmission (Bezara *et al.* 2020). One of the highest expressions of both ACE2 and transmembrane protease serine 2 (TMPRSS2), a cofactor for SARS-CoV-2 entry, is in ATII cells (Ziegler *et al.* 2020, Carcaterra and Caruso 2021). In the lung parenchyma, ACE2 was found on the apical surface of a small subset of ATII cells and colocalized with TMPRSS2. About 83 % of ACE2-expressing cells are ATII cells and 5 % are ATI cells (Lukassen *et al*. 2020). ACE2-expressing ATII cells had high levels of multiple viral response-related genes, supporting the role of these cells in viral infection and control (Ziegler *et al.* 2020). Over 34 % of ATII cells express *TMPRSS2*, 1.4 % express *ACE2*, and 0.8 % co-express *TMPRSS2* and *ACE2*. This is in accordance with the report of Zou *et al.* (2020) on the average proportion of ACE2-positive ATII cells of about 1 %.

Other receptors expressed at ATII cells may also be involved in the development of COVID-19. Recently, it has been proposed that the spike protein of SARS-CoV-2 has a strong interaction with TLR4, an innate immune receptor on the cell surface that recognizes pathogen-associated molecular patterns including viral proteins (Aboudounya and Heads 2021). In physiological conditions, two minor anionic phospholipids present in the pulmonary surfactant, palmitoyl-oleoyl-phosphatidylglycerol (POPG) and phosphatidylinositol (PI), block TLR2 and TLR4 in the lungs (Kuronuma *et al*. 2009, Voelker and Numata 2019). Therefore, infection of ATII cells with SARS-CoV-2 and the following lack of surfactant production may expose TLR4 on alveolar cells for the virus and promote inflammation and lung injury (Aboudounya and Heads 2021).

Regulation of ACE2 expression on ATII cells

COVID-19 tends to progress faster in elderly people (Vašků 2020). Interestingly, age, sex, and comorbidities do not increase ACE2 protein expression in the human respiratory tract (Bezara *et al.* 2020). While the expression of ACE2 and TMPRSS2 genes in the ATII cells in elderly and young patients are comparable, 263 different genes are downregulated in cells from the elderly, with superoxide dismutase 3 (*SOD3*), an important antioxidant enzyme, being most downregulated (Abouhashem *et al*. 2020). In patients on mechanical ventilation in comparison to non-ventilated individuals, ACE2 was strongly upregulated with age, which was associated with prominent expression in ATII cells. These findings provide a potential mechanism for a more severe course of COVID-19 in the elderly (Paces *et al*. 2020, Baker *et al*. 2021).

ACE2 receptors are activated by interferons, which enhances infection (Ziegler *et al*. 2020). It is proposed that the development of a such “hyperactive” immune response plays a role in the evolution of the disease. The presence of pan JAK/STAT components in ATII cells suggests that ACE2 is also activated by other cytokines (Hennighausen and Lee 2020). ACE2 has

different expression levels in airways under distinct chronic inflammatory airway diseases, such as chronic obstructive pulmonary disease (COPD) and allergic asthma, which may affect the management of primary airway diseases in patients with COVID-19 (Yao *et al*. 2020). The expression of ACE2 in ATII cells is modulated by pollutants or cigarette smoke (Liu *et al.* 2021). Elevated oxygen levels did not affect the expression of ACE2 in cell culture but did increase the gene and protein expression of *TMPRSS11D*. Thus, oxygen supplementation can be a potential risk factor for COVID-19 (Myti *et al*. 2020).

The models to study ATII cells and COVID-19

Representative models to study virus vs. host interactions in the lungs in detail are not available. These models should be based on human cells relevant to the disease in order to study the behavior of SARS-CoV-2 and possible pharmacological interventions. In general, SARS-CoV-2 infection of human lung epithelia can be modelled by two basic methods: using stem cell technology and organoids. Human 2D and 3D cultures of differentiated cells and organoids allow for modelling of the structure and physiology of different tissues and organs (Trevisan *et al*. 2021). These *in vitro* cell systems are generated from induced pluripotent stem cells (iPSCs), primary cells, cell lines, and *ex vivo* tissue biopsies (Leibel *et al.* 2020, Han *et al*. 2021, Lamers *et al*. 2021, Leibel *et al.* 2021). ATII cells must retain their typical features, including the ability to self-renew, produce surfactants, and differentiate into ATI cells (Katsura *et al*. 2020). In preclinical studies, A549 cells are often used as a model for ATII cells. They do not seem convenient for this purpose because they come from human lung cancer-derived cell lines and lack the expression of important lung epithelial genes including several genes coding surfactant proteins. On the other hand, primary alveolar type II cells express *SFTPA1*, *SFTPA2*, *SFTPC*, and *SFTPD* as well as *ACE2* and *TMPRSS2*, two genes encoding host cell proteins essential for SARS-CoV-2 cell entry (Abo *et al.* 2020).

Morphological changes of alveolar type II cells

The visualization of the ATII cells changed by SARS-CoV-2 infection helps to uncover the pathophysiology of respiratory failure. The majority of lung specimens are investigated *post mortem* and diffuse alveolar damage (DAD) in COVID-19 patients is the most relevant histologic pattern. The findings are usually equivalent to the pattern of alveolar epithelial injury of other etiology (Ochs *et al.* 2021). In lung sections from COVID-19 patients, the predominant feature was DAD with fibrosis in the final stage of illness (Santana *et al*. 2021); some patients showed focal pulmonary microthrombi (Bradley *et al.* 2020). In other autopsy and microscopy studies of deceased COVID-19 patients, the most important aspect was the focal cytopathic changes of ATII cells. Enlargement of ATII cells was accompanied by rich cytoplasm and enlarged irregular nucleus with a single-centered prominent nucleolus and inclusions situated at the periphery of the nucleus. In some areas, ATII cells tended to form clusters marked as multinucleated giant cell-like alveolar epithelial cell aggregates (Oprinca and Muja 2021). In general, all findings support cytokine storm and severe fibrosis in the lungs of COVID-19 patients (Chen *et al.* 2021).

The *post mortem* findings may differ from those of the earlier stages of the disease as shown by *ante mortem* investigation. Morphologically distinct features in early stages of COVID-19 pneumonia, with epithelial and endothelial cell abnormalities different from either classical interstitial lung diseases or diffuse alveolar damage, were shown by transbronchial lung cryobiopsy in twelve COVID-19 patients with moderate COVID-19 pneumonia. Within 20 days of symptom onset, this investigation revealed spots of patchy acute lung injury with ATII cell hyperplasia, with no evidence of hyaline membranes. Over 50 % of ATII cells had strong nuclear expression of phosphorylated STAT3 proteins, involved in cytokine signaling (Doglioni *et al.* 2021). ATII cell hyperplasia was a prominent event in the majority of cases and may be considered an essential part of the epithelialization process in wound healing (Ruaro *et al.* 2021).

Using transmission electron microscopy, Ochs *et al.* (2021) suggested a sequence of events leading to the reduction of alveolar volume and alveolar gas exchange area. It begins with alveolar epithelial cell damage followed by surfactant dysfunction, alveolar instability, and microatelectasis. The process of collapse induration is further based on basal lamina denudation, collapse and sealing of the alveoli by proliferating alveolar epithelial cells, and the forming of thickened septa. This provides clear evidence of surfactant dysfunction and alveolar instability in fibrosis initiation, as well as evidence of early surfactant dysfunction as an important event in the pathophysiology of COVID-19.

These, together with other data (e.g. Busani *et al.* 2020, Koumbourlis and Motoyama 2020) suggest that exogenous surfactant therapy may restore the surfactant system and prevent lung fibrosis in COVID-19 patients.

Surfactant and ATII cells in SARS-CoV-2 infection: Implications for the pathophysiology of ARDS

The lung injury in COVID-19 usually begins after an asymptomatic phase when the infection is not combatted by nasal mucosa (Pedan *et al.* 2020) and continues as a mildly symptomatic phase after involvement of the epithelium of the larger airways (Mason 2020). Consequently, SARS-CoV-2 infection of the ACE2-receptor bearing cells in the peripheral lungs induces a local immune response and may lead to severe lung damage (Paces *et al.* 2020), resulting in acute respiratory distress syndrome (ARDS). In general, the pathophysiology of ARDS is very complex (Otáhal *et al*. 2016, Mokrá 2020). New coronavirus-induced ARDS has several specific features. It affects mainly lungs, with minor damage to other organs. Its main characteristic is the dissociation between the severity of the hypoxemia and the maintenance of relatively good respiratory mechanics (Gattinoni *et al*. 2020). Sudden opening of a previously undetected probe-patent foramen ovale could explain this finding. Opening of such an intracardiac shunt would not worsen lung mechanical properties. The evidence of hypercoagulability and pulmonary embolisms at autopsy support this hypothesis, as they would contribute to increased pressure in the pulmonary arterial bed and the right heart, potentially leading to opening of a short circuit (Fisher 2020).

The lack of surfactant leads to increased surface tension, alveolar flooding, and atelectasis, resulting in ineffective gas exchange and respiratory failure (Mason 2020). The clinical manifestation may be relatively mild in some COVID-19 patients and the clinical symptomatology is inconsistent with the degree of laboratory and imaging findings (Li and Ma 2020). The timing of onset of COVID-19-related ARDS is about 8-12 days in contrast to ARDS Berlin criteria, which give 7 days as the onset limit (ARDS Definition Task Force 2012).

The main cause of COVID-19-related ARDS is injury to ATII cells resulting in altered surfactant production and lung fluid homeostasis (Morris *et al.* 2020). Viral infection of ATII cells activates the aberrant protein pathways followed by abnormal cross-talk between infected ATII cells and noninfected endothelial cells and induces the relaxation and dilation of pulmonary vessels. Finally, alveolar epithelial and endothelial cells die by pyroptosis and necroptosis (Morris *et al.* 2020). Activation of alveolar macrophages in close proximity to ATII cells contributes to overactivation of the inflammatory immune response, which can lead to a cytokine storm and subsequent immune exhaustion (Morris *et al.* 2020, Paces *et al.* 2020, Chen *et al.* 2021, Chilosi *et al.* 2021).

Viral proteins can target both transcription and epigenetic factors, which, in turn, modulate important members of the surfactant metabolism process, including *SFTPB*, *SFTPC*, and S*FTPD* genes (Islam and Khan 2020). Surfactant metabolism is modulated not only by viral proteins, but also through irregular host responses. Unaffected metabolism in ATII cells is crucial for surfactant synthesis. Its disruption could contribute to the pathogenesis of virus-induced ARDS, as it is associated with reduced levels of liponucleotides, essential precursors to *de novo* phospholipid synthesis in ATII cells. Altered surfactant metabolism may be reversed by diet, able to influence the lipidome of ATII cells (Rosas *et al.* 2021).

As mentioned above, the primary histological manifestation of severe lung disease in COVID-19 patients is diffuse alveolar damage with no distinctive morphological features with which to confidently differentiate COVID-19-related DAD from DAD due to other causes (Konopka *et al.* 2020). DAD includes an acute phase with alveolar epithelial injury, surfactant changes, and edema formation. Capillary congestion, platelet adhesion, fibrin thrombi, and rupture of the capillary walls complete the image. Because of the increased permeability of the alveolar-capillary barrier followed by transudation of plasma proteins, formation of hyaline membranes, and an inflammatory exudate, characteristic of ARDS, it is also called the exudative phase (Mason 2020). The late organizing phase with cuboidal metaplasia of ATII cells and thickening of inter-alveolar septa, ultimately resulting in either restoration or fibrosis, is also called proliferative (Ochs *et al.* 2021, Santana *et al.* 2021). It is clearly evidenced that surfactant alterations resulting from an initial alveolar epithelial injury lead to alveolar instability and collapse, followed by the unfolding and denudation of alveolar epithelial basal laminae and re-epithelialization (Ochs *et al.* 2021, Santana *et al.* 2021). Structural changes of the lungs and

surfactant dysfunction affecting tissue mechanical behavior are similar to changes seen in another lung injury. Extensive alveolar microangiopathy further contributes to mechanical changes in the tissue (Dimbath *et al.* 2021).

In COVID-19 related ARDS, ATII cells infected by the virus may lose their ability for normal repair and contribute to the fibroproliferative reaction. This happens through the secretion of growth factors and proinflammatory molecules, which can finally result in lung fibrosis as a background for interstitial lung diseases (Mason 2020, Ruaro *et al.* 2021).

Exogenous surfactant treatment in COVID-19 patients

To date, no treatment has emerged as being significantly effective in patients with respiratory failure due to SARS-CoV-2 infection. Surfactant replacement therapy has been a routine part of the management of neonatal respiratory distress syndrome (RDS) over several decades. It improves the oxygenation and survival of babies with primary surfactant deficiency of prematurity (Herting *et al.* 2020). This treatment has also been tested for acute respiratory distress syndrome (ARDS). Even if some patients have benefitted from surfactant administration, several studies had to be terminated without convincing success, possibly due to the complexity of this syndrome (Schousboe *et al.* 2020, Veldhuizen *et al.* 2021). The pathomechanisms of COVID-19-induced ARDS are based on damage to ATII cells. The course of ARDS in COVID-19 patients is atypical and shows a discrepancy between severe hypoxemia and the relatively well-preserved mechanical behavior of the lungs, which makes it more similar to neonatal RDS (Koumbourlis and Motoyama 2020).

It is essential to understand the central role of pulmonary surfactant deficiency in SARS-CoV-2-induced ARDS. It is justified to consider exogenous surfactant therapy as a rational and well-founded treatment in COVID-19 ARDS patients for the following reasons.

Administration of exogenous surfactant increases the surfactant pool in the lungs, which is reduced due to the damage of ATII cells and/or by the development of inflammation. It also restores alveolar-capillary barrier and, thus, reduces edema (Mirastschijski *et al.* 2020). Exogenous surfactant reduces inflammation by multiple mechanisms. Exposure of TLR4 in the lungs due to reduced pulmonary surfactant makes the receptors available for the SARS-CoV-2 to bind to and activate TLR4 to increase ACE2 expression, facilitating entry and causing hyperinflammation (Aboudounya and Heads 2021). By blocking TLR4, surfactant hinders this receptor-pathogen interaction (Kuronuma *et al.* 2009, Abate *et al.* 2010). Some surfactant phospholipid species may inhibit the initiating step of the pro-inflammatory signaling pathways and also inhibit inflammatory sequelae associated with virus infections (Voelker and Numata 2019). Surfactant may restore immune homeostasis, as it is a strong defender against the virus itself (Takano 2020). Surfactant lipids directly disrupt the virus particles binding to host cell plasma membrane receptors, required for viral uptake (Bollag and Gonzales 2020).

Reduction of surface tension in the alveoli and small airways after surfactant administration improves the gas exchange and mechanical behavior of the lungs, which is followed by an increase in oxygenation. Finally, the patient may benefit from early administration of pulmonary surfactant by the shortening of the duration of mechanical ventilation and enhanced weaning (Mirastschijski *et al.* 2020, Cattel *et al.* 2021). To achieve a synergistic effect, exogenous surfactant can be combined with anti-inflammatory, anti-oxidant, anti-viral, and anti-bacterial agents such as, e.g. ambroxol, which, in addition, has a direct impact on the production and secretion of the surfactant from the ATII cells (Kumar 2020).

Taken together, these data lead to a rational approach in the treatment of the disease by the administration of exogenous surfactant. Such treatment has the potential to contribute efficiently to the repair of damaged alveoli and to prevent the severe respiratory failure associated with SARS-CoV-2 infection. The success of such a treatment was documented by several case studies and encouraged the medical community to perform large international clinical phase 2 studies which are under way.

Exogenous surfactant therapy is also effective in very severe courses of COVID-19. A 48-year-old male non-smoker with COVID-19-related ARDS did not improve on ECMO for 5 days. On day 11 of hospitalization, he was administered five vials of 6 ml surfactant (calfactant; 35 mg/ml phospholipid suspension, 20 mg phospholipids/kg of body weight). Surfactant was administered *via* a tracheobronchial suction catheter passed through the endotracheal tube with the distal

suction tip positioned above the carina, and then dispersed directly into the lungs during positioning of the patient. Based on improved oxygenation, the patient was weaned from ECMO after 36 h, extubated the following day, and discharged 8 days later (Heching *et al*. 2021). The benefits of this treatment have also been shown by the retrospective analysis of data from seven COVID-19 ARDS patients receiving off-label exogenous surfactant poractant alfa at a dose of 720 mg in 150 ml physiological solution, divided into five 30 ml aliquots. Surfactant was delivered *via* a bronchoscope into second-generation bronchi. The main finding was a trend toward the reduction of 28-days mortality within the surfactant group. Surfactant delivery through bronchoscopy has been suggested as being feasible, well-tolerated, and safe for COVID-19 ARDS patients and health care providers during the pandemic (Piva *et al.* 2021). Using computational fluid dynamics to simulate exogenous surfactant instillation with 3D human airway models and observing how it moves in the liquid layer covering the airway wall and reaches alveolar regions, it was concluded that selective wedge instillation under bronchoscopic observation should be tried for COVID-19 pneumonia before the onset of ARDS, which may also be useful for preventing secondary lung fibrosis (Kitaoka *et al.* 2021).

A single-center study in adult COVID-19 patients with moderate to severe ARDS requiring auxiliary respiratory devices is on the way. The exogenous surfactant beractant, bovine lung extract, is given in a standard-dose 4 ml suspension containing 100 mg phospholipids for an adult person with about 70 kg of body weight (b.w.). The first dose of surfactant is given intratracheally on the day of intubation and the second dose 6 h later. The main outcomes are 30 days mortality, mortality during stay in the intensive care unit (ICU) up to 30 days, an ICU stay of up to 30 days, and time under mechanical ventilation up to 30 days (Dabbagh *et al.* 2020).

At https://clinicaltrials.gov, seven studies using exogenous surfactant administration in adult patients with COVID-19 ARDS and designed mostly as clinical phase 2 studies are registered. Two studies use modified porcine surfactant poractant alfa. One study called "Curosurf® in Adult Acute Respiratory Distress Syndrome Due to COVID-19 (Caards-1)" is carried out in France. The patients receive either the bronchial fibroscopy alone (to aspirate the secretions) or a bronchial fibroscopy with administration of modified porcine surfactant poractant alfa at a dose of 3 ml/kg b.w. diluted to 16 mg/ml and distributed into each of the 5 lobar bronchi (https://clinicaltrials.gov/ct2/show/NCT04384731). Another study using the same exogenous preparation is called "Poractant Alfa – Curosurf and SARS-COV-19 ARDS (COVID-19)" is being performed in the UK with 85 participants. The efficacy and safety of poractant alfa will be evaluated in terms of ventilatory-free days during the 21 days after randomization, in adult patients with ARDS due to SARS-CoV-2 infection. Patients receive three administrations with a 24-hour dosing interval; endo-tracheal administration 1, 2, and 3 will consist of poractant alfa bolus: 30 mg/kg b.w. (0.375 ml/kg b.w.) (https://clinicaltrials.gov/ct2/show/NCT04502433). In "A Clinical Trial of Nebulized Surfactant for the Treatment of Moderate to Severe COVID-19 (COVSurf)", patients will be administered surfactant *via* the COVSurf Drug Delivery System or will receive regular Standard of Care treatment. This study evaluates the feasibility, safety and efficacy of nebulized surfactant in adult COVID-19 patients requiring mechanical ventilation for respiratory failure; the study is intended as a dose-escalating randomized open-label clinical trial of COVID-19 patients (Dushianthan *et al.* 2020, https://clinicaltrials.gov/ct2/show/NCT04362059). In "London's Exogenous Surfactant Study for COVID19 (LESSCOVID)," patients with COVID-19-induced respiratory failure will be randomly assigned to receive either standard treatment or standard treatment plus bovine lipid extract surfactant (BLES). BLES is administered in doses of 50 mg/kg b.w. at a concentration of 27 mg/ml. The material will be instilled as soon as possible *via* the suction catheter through the endotracheal tube. The procedure will be repeated at 24 and 48 h during intubation, so the patient will receive up to 3 doses (https://clinicaltrials.gov/ct2/show/NCT04375735). The study "Surfactant-BL in Adult Acute Respiratory Distress Syndrome Due to COVID-19," performed in Russia, includes 4 different cohorts of patients who inhaled surfactant emulsion Surfactant-BL (Biosurf LLC, Russia) at 150 mg every 12 h on the first, second, third, fourth, and fifth days of the treatment period, inclusive. Primary outcome measure is the mean duration of oxygen therapy (days) in the treatment group and in the control group (https://clinicaltrials.gov/ct2/show/NCT04568018). Clinical trial "Exogenous Surfactant Through Nebulizer Mask on Clinical Outcomes in COVID-19 Patients (CovidSurf)" is aimed to evaluate the effect of exogenous nebulized surfactant in the pre-intubation stages of the

disease. Nebulized surfactant would be administered by face mask, which has a nebulizer. The type of surfactant preparation is not listed (https://clinicaltrials.gov/ct2/show/NCT04847375). The patients in "The Safety and Preliminary Tolerability of Lyophilized Lucinactant in Adults With Coronavirus Disease 2019 (COVID-19)" study receive synthetic surfactant lucinactant (KL4-surfactant) as a liquid at a dose of 80 mg total phospholipids/kg b.w. Primary outcome measures are the safety and feasibility of lucinactant therapy in treating COVID-19 and oxygen index through 12 h post initiation of dosing (https://clinicaltrials.gov/ct2/show/NCT04389671).

Conclusions

SARS-Cov-2 enters *via* the ACE2 receptors of the ATII cells, producers of lung surfactant. Surfactant lipids and specific proteins protect the respiratory system against pathogens, including the novel coronavirus. Injury to the ATII cells considerably reduces the surfactant alveolar pool and leads to various respiratory symptomatology, including ARDS. Thus, the clinical manifestations of the pulmonary form of COVID-19 is a direct consequence of the involvement of ATII cells and surfactant deficiency. Clinical data provide rationale for using exogenous surfactant as a valid supportive treatment in COVID-19 patients. The potential of surfactant as being anti-inflammatory and lung-protective therapy is influenced by several variables, including the way of delivery, timing, dosing, and surfactant preparation as well as mechanical ventilation. Pulmonary surfactant as a target for SARS-CoV-2 infection can become a means for suppressing the consequences of this infection.

Conflict of Interest

There is no conflict of interest.

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