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Exogenous pulmonary surfactant: A review focused on adjunctive therapy for severe acute respiratory syndrome coronavirus 2 including SP-A and SP-D as added clinical marker

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Abstract

Type I and type II pneumocytes are two forms of epithelial cells found lining the alveoli in the lungs. Type II pneumocytes exclusively secrete ‘pulmonary surfactants,’ a lipoprotein complex made up of 90% lipids (mainly phospholipids) and 10% surfactant proteins (SP-A, SP-B, SP-C, and SP-D). Respiratory diseases such as influenza, severe acute respiratory syndrome coronavirus infection, and severe acute respiratory syndrome coronavirus 2 infection are reported to preferentially attack type II pneumocytes of the lungs. After viral invasion, consequent viral propagation and destruction of type II pneumocytes causes altered surfactant production, resulting in dyspnea and acute respiratory distress syndrome in patients with coronavirus disease 2019. Exogenous animal-derived or synthetic pulmonary surfactant therapy has already shown immense success in the treatment of neonatal respiratory distress syndrome and has the potential to contribute efficiently toward repair of damaged alveoli and preventing severe acute respiratory syndrome coronavirus 2–associated respiratory failure. Furthermore, early detection of surfactant collectins (SP-A and SP-D) in the circulatory system can be a significant clinical marker for disease prognosis in the near future.

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COVID-19, SARS-CoV-2, ARDS, Pulmonary surfactant, Collectin.

Abbreviations

acute respiratory distress syndrome, ARDS; angiotensin-converting enzyme 2, ACE2; coronavirus disease 2019, COVID-19; dipalmitoyl-phosphatidylcholine, DPPC; human immunodeficiency virus, HIV; interleukin, IL; palmitoyl-oleoyl-phosphatidylglycerol, POPG; phosphatidylinositol, PI; respiratory distress syndrome, RDS; severe acute respiratory syndrome coronavirus 2, SARS-CoV-2; surfactant proteins, SP; Toll-like receptor, TLR; tumor necrosis factor, TNF.

Introduction

According to the World Health Organization, ‘ten threats to global health in 2019’ were air pollution and climate change, noncommunicable diseases, a global influenza pandemic, fragile and vulnerable settings, antimicrobial resistance, Ebola virus and other high-threat pathogens, weak primary health care, vaccine hesitancy, dengue, and HIV [1]. Now, in addition to these threats, the prominence of the coronavirus disease 2019 (COVID-19) pandemic demands an urgent multidisciplinary strategy to control disease spread and prevent its complications. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) primarily attacks the respiratory system. As the disease progresses, it may cause no symptoms, that is, in asymptomatic individuals, mild symptoms such as a cough and rhinitis or severe symptoms such as hypoxia, dyspnea, pneumonia, and so on. More severely, patients develop interstitial inflammation, pulmonary infiltrates, a massive cytokine storm, and, consequently, acute respiratory distress syndrome (ARDS), leading to respiratory failure. Nearly

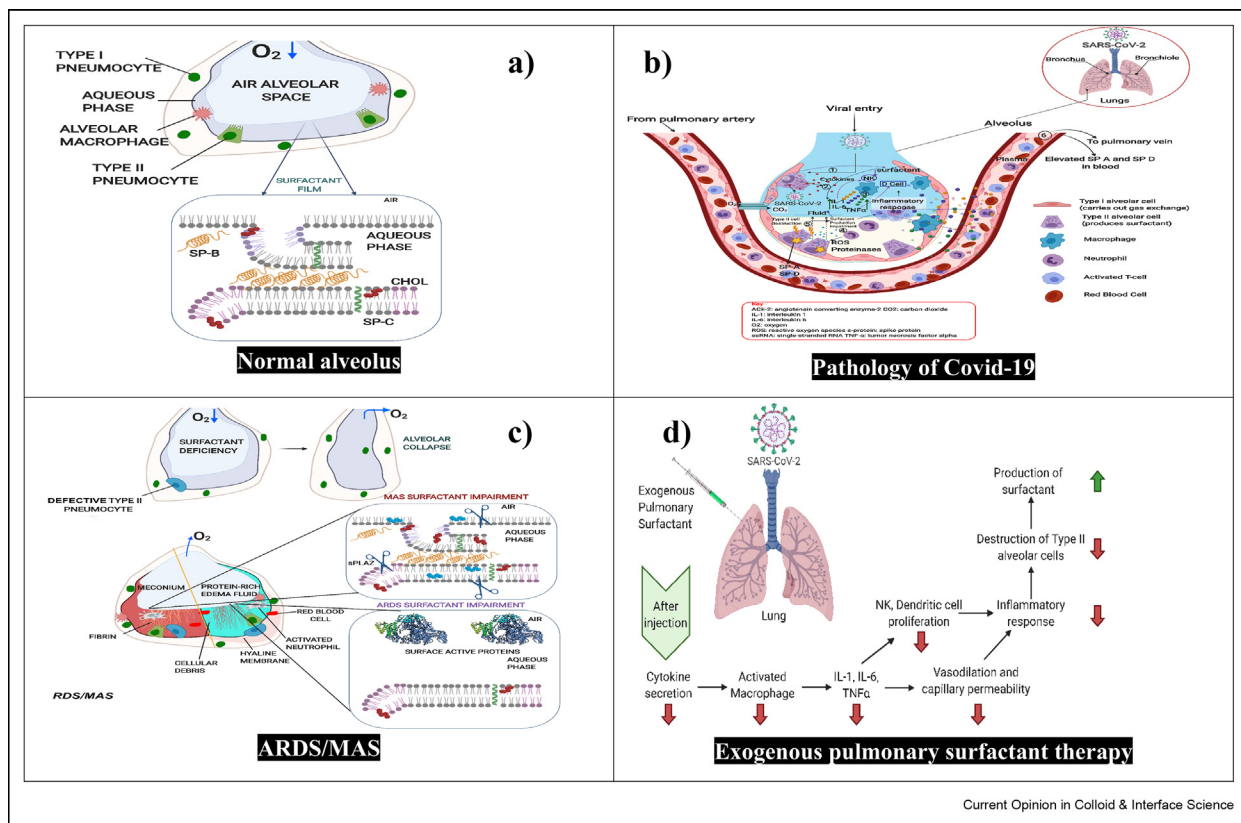
20% of patients experience the severe form (stage 3) [2].

Deep into lung physiology: the alveolar epithelium

Alveoli are the crucial extremity of the distal lung and work in gaseous exchange between the bloodstream and inhaled air. Type I pneumocytes and type II pneumocytes, two forms of epithelial cells, are found lining the alveolus. Among type I pneumocytes (broad and flat), 95% surround the surface area and are involved in gaseous exchange. Type II pneumocytes are cuboidal, make up the rest of the alveolus, and exclusively secrete ‘pulmonary surfactants’ from typical organelles called lamellar bodies [3]. Pulmonary surfactants are a type of

lipoprotein complex made up of 90% lipids (majorly phospholipids) and 10% surfactant proteins (SP-A, SP-B, SP-C, SP-D) [4,5]. Pulmonary surfactant phospholipids (mainly dipalmitoylphosphatidylcholine [DPPC]) in the alveolar lumen decrease the surface tension on the walls of alveoli from 70 mN/m to 1 mN/m [6]. SP-B stabilizes the lipid coating during respiration, whereas SP-C works on compression of the lipid coating, attuned with decompression during inspiration. By the end of expiration, the surface tension should be <2 mN/m to prevent alveolar collapse [7]. Hence, by decreasing the surface tension during inspiration and inhibiting alveolar collapse after expiration, pulmonary surfactants act as an efficient lifesaver (Figure 1a).

Figure 1



Scheme of alveolar collapse due to COVID-19–mediated surfactant impairment and the role of exogenous pulmonary surfactant therapy. (a) Normal alveolus. (b) Pathophysiology of SARS-CoV-2 infection. After entry of SARS-CoV-2 in type II alveolar cells (1), the infected cell becomes defective for surfactant production and releases cytokines (2), which in turn activates alveolar macrophages (3) to release IL-1, IL-6, and TNF- α , and the level of these proinflammatory molecules rises; these molecules induce differentiation of natural killer (NK) cells and dendritic (D) cells, causing release of more proinflammatory response; cumulative effect leads to prolonged inflammatory response, and increased vasodilation causes influx of neutrophils and activated T cells in the alveolus from the capillary tube. The neutrophil produces ROS and proteinases (4), leading to further destruction of healthy type II cells (5); as a result, surfactant production decreases markedly, which in turn causes fluid accumulation in the alveolus leading to alveolar collapse and ARDS further; destruction of type II cells leads to leakage of SP-A and SP-D in the capillary, and their concentration becomes elevated in blood (6). (c) Alveolar collapse leading to the development of ARDS and MAS. (d) The probable mechanism of exogenous pulmonary surfactant therapy for pulmonary protection. ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; IL, interleukin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNF- α , tumor necrosis factor alpha; ROS, reactive oxygen species; MAS, macrophage activation syndrome.

Biophysical role of pulmonary surfactants

Surface tension (γ) is the amount of energy required to conquer the forces that reduce the area exposed to a medium excluding the liquid by increasing its surface over a unit of area and articulated as energy per unit of length or area (mN/m or J/m^2) [8]. The force of attraction between liquid molecules generates γ , which acts in all direction in the liquid bulk phase. Nevertheless, such a force of attraction is not fully compensated, resulting in a net attractive force toward the interior of the liquid. A tiny layer of water covers up the lung epithelium, and these forces provide mechanical and structural stability to the lungs while breathing. The failure to limit these forces during exhalation causes the smallest alveoli more susceptible to collapse. The area exposed to the air reduces owing to γ , which subsequently reduces the area accessible for gaseous exchange [9–11].

The surfactant produced by alveolar type II cells minimizes these surface forces by insertion of phospholipid components at the interface, reducing surface tension by virtue of displacement of water molecules aside from the interface. The phospholipids occupy the entire interface, eventually reaching a state of surface tension equilibrium (γ_{eq}), although the occupied alveolar surface area varies continuously as per the breathing cycle throughout exhalation and inhalation. The surfactant prevents alveolar collapse via minimizing the surface tension below equilibrium to a minimal value (γ_{min}) owing to its specific phospholipid composition enriched in DPPC, allowing effective packing by its disaturated acyl chains.

It is hypothesized that, during respiration, interfacial compression leads to a continuous modification of the interfacial surface. This causes more fluid lipid components, such as unsaturated phospholipids and cholesterol to squeeze out from the interfacial surface [8,12–14]. This type of lipid refinement favors DPPC enrichment at the interface, resulting in a tightly packed surface membrane to achieve surface tensions near 0 mN/m . Low surface tensions reflect the ability of the surfactant to stabilize the lung during exhalation. Conversely, during lung re-expansion or inhalation, the surface area of the interface increases, and high surface tensions of the expanding aqueous hypophase are minimized by the reinsertion of the surfactant at the interfacial film from the multilayer reservoir below. In a healthy lung, surface tensions achieved during re-expansion should be lower than $\sim 22\text{--}25 \text{ mN/m}$ [14,15], which is proposed as equilibrium surface tension and is accomplished by efficient reinsertion of the active surfactant back into the surface.

According to Laplace's law, alveolar stability is achieved owing to the low surface tension. Laplace's equation

($\Delta P = 2\gamma/R$, where P indicates the closing pressure of an ideal spherical chamber, γ is the surface tension, and R is the radius) suggests that at a given pressure, it is quite impossible for coexistence of two linked alveoli with variable radii and the same surface tension. The inner pressure of the smaller alveolus would make it vulnerable to collapse into the larger one. The stability of the lungs is highly favored by the surfactant, which is crucial for decreasing surface tension with respect to alveolar size [16]. Consequently, impaired surface activity causes increased alveolar surface tension and eventually results in alveolar instability and atelectasis. Thus, increasing surface tension may affect normal biological function of the lungs and lead to notable decrease in lung compliance [17].

Lung pathology due to SARS-CoV-2 infection

SARS-CoV-2 travels from the nasal cavity to the gaseous exchange units of the lungs. Respiratory diseases such as influenza and SARS-CoV infection have been reported to preferentially attack type II pneumocytes of the lungs, and viral release followed by replication directs the cells into apoptosis and eventually death [2]. In particular, SARS-CoV-2 is able to affect type I pneumocytes, type II pneumocytes, and alveolar macrophages [18], resulting in dysfunctional lungs and increased morbidity worldwide. After viral invasion, consequent viral propagation and destruction of type II pneumocytes causes altered surfactant production, resulting in dyspnea and ARDS in patients with COVID-19, and in more severe cases, the massive destruction of this 'alveolar defender,' namely, type II pneumocytes, leads to respiratory failure (Figure 1b and c). Fifty-three percent of mortality due to COVID-19 ARDS is because of respiratory failure [19]. Nevertheless, pulmonary surfactants also work in host defense against viral infection. Surfactant liquid interfaces served as a protective barrier to exclude virus invasion via improving mucociliary transport mechanism. Furthermore, surfactant components directly interact with the structural machineries of respiratory viruses inhibiting their proliferation through provoking agglutination, inactivation, and opsonization [15,20,21]. While the surfactant phospholipid DPPC maintains the respiratory mechanics by reducing surface tension at the air–tissue interface of the alveolar compartment, as mentioned previously, the phospholipids phosphatidylinositol and palmitoyl-oleoyl-phosphatidylglycerol disrupt Toll-like receptor signaling to suppress the secretion of proinflammatory cytokines such as tumor necrosis factor alpha and interleukins (ILs; IL-6 and IL-8) [7]. Collectins (SP-A and SP-D) act in host immune response, followed by pathogen invasion. The exclusive interaction of the C-terminal lectin domain of collectins with surface oligosaccharides of viruses is reported, which enhances viral clearance mediated by

macrophages and monocytes. Besides, SP-A, as the immunomodulator, can suppress dendritic cell maturation and inhibit eosinophils from releasing excessive IL-8 [7]. Surfactant proteins are well reported in the host defense mechanism against several pathogens [20–22]. For instance, in the case of influenza A virus, collectins inhibit surface proteins and neutralize the virus, wherein heavily glycosylated ones are the most sensitive to SP-A [23]. Notably, a study on purified SARS-CoV S-protein was reported to be bound by SP-D in the presence of Ca^{2+} ions and to activate lung macrophages [24]. Furthermore, the ACE2 mode of entry for both SARS-CoV-2 and SARS-CoV is proven and documented up to now. Interestingly, SARS-CoV ACE-2 receptor expression strikingly varies in type II pneumocytes in patients with different SARS-CoV infection. Reduced surfactant production leads to an increase in SARS-CoV vesicular release from the apical portion of the cells [24]. Variation in receptor expression may conclude the explanation of varying susceptibility to SARS-CoV and SARS-CoV-2 in patients.

Exogenous surfactant therapy: a potential intervention

No specific and effective treatment has yet been discovered for COVID-19, and many drugs are being repurposed for ongoing hospital cases. Indeed, a number of supportive treatments are needed, especially for critically ill patients who develop ARDS. Decreased lung compliance is a typical feature of ARDS, which is often based on the anarchy of endogenous surfactant biology. Patients need oxygenation-enhancing and pulmonary edema—supportive treatments owing to excessive inflammation of the lung, as evidenced from lung autopsies. COVID-19 mortality is as high as 88% in the ventilation group [18]. Remarkably, exogenous animal-derived pulmonary surfactants (from cattle and pigs) or synthetic surfactant therapy has already presented immense success in the treatment of neonatal respiratory distress syndrome (RDS). The World Health Organization previously recommended surfactant replacement therapy for neonatal RDS [25]. Ainsworth et al analyzed 199 surfactant-deficit neonates with intubation of poractant alfa (an animal-sourced surfactant) and pumactant (synthetic surfactant), wherein the mortality rate was less in the natural surfactant group [26]. And it considerably reduced morbidity, and it decreased the occurrence of air leak in neonates. Several studies have shown the crucial role of exogenous surfactants as an immune regulator in pediatric patients with functional therapeutic potential. Although surfactants showed clear effect in neonatal patients, there is scanty evidence in adult patients in terms of their doses and administration methods, resulting in poor outcomes [27, 28]. ARDS in adults usually causes more complication ensuing loss of the pulmonary endothelium and epithelium cells,

sophisticated etiology, and chaotic immune system; simple surfactant supply is not enough for adult patients with ARDS [29]. The most common cause of death in patients with ARDS is multiorgan system failure rather than respiratory failure. Surfactant administration helps to improve ARDS, but is not solely sufficient for treating adult patients with ARDS owing to several factors such as causes, severity, and immune responses of patients. SP-A, SP-B, SP-C, and SP-D surfactant proteins have their own crucial role in physiological activity. The hydrophobic proteins SP-B and SP-C were responsible for lowering of γ , whereas SP-A and SP-D are hydrophilic proteins possessing a centralized role in host defense [30]. It is assumed that the presence or absence of such proteins along with other components in the surfactant may change the effectiveness of the therapy. However, not all is gloom and doom in case of surfactant therapy in ARDS. Lewis and Jobe [31] earlier reported positive results, improved gaseous exchange, and a decreased mortality, suggesting future development of optimal surfactant delivery techniques and exogenous surfactant preparations. An optimized exogenous surfactant in combination with precise components for mimicking the natural pulmonary surfactant may open up a new avenue into improving patients with COVID-19. In the case of meconium aspiration syndrome, which is pathophysiologically very similar to COVID-19 respiratory distress, early application of surfactant therapy exhibited positive results [32]. In meta-analysis, exogenous surfactants improved oxygenation [33]. The amount of the first surfactant dose is thought to be more crucial than the source. In addition, a higher first dose lowers the need for repetitive (at least second and third) doses [34].

The exogenous surfactant exhibits anti-inflammatory properties and reduces the production of tumor necrosis factor alpha, IL-1, IL-6, and so on (notably elevated during COVID-19) (Figure 1d). It reduces inflammation after intratracheal application to the lung and topical application on the skin. Herein, the exogenous surfactants can greatly lower the proinflammatory gene expression in the skin wounds [32]. Thus, it may efficiently contribute toward repair of damaged alveoli and prevent SARS-CoV-2—associated respiratory failure. Corey et al. have stressed the need for further investigation regarding exogenous surfactant therapy [34]. A third-generation synthetic surfactant named CHF5633, comprising analogs of SP-B and SP-C (0.2% and 1.5%, respectively) in the 1:1 DPPC:palmitoyloleoylphosphatidylglycerol mix, exhibited an encouraging outcome in a phase I clinical trial designed for RDS [39]. Moreover, CHF5633 has the potential to reduce synthesis of proinflammatory cytokines in human macrophages, and it may enhance the role of exogenous surfactant therapy in severe lung diseases. A clinical trial on exogenous surfactant administration therapy (bovine lipid extract) for patients with COVID-19 and ARDS named

'LESSCOVID' has begun at London Health Sciences Centre, UK [35]. Another nebulized surfactant 'COVsulf' is being clinically trialed at University Hospital Southampton NHS Foundation Trust [36]. Some pharmacological companies such as Windtree Therapeutics™ (synthetic KL4 surfactant) and Lyomark Pharma GmbH (bovactant surfactant) have been working toward clinical studies with their potential therapeutics for patients with COVID-19 [32].

Furthermore, SP-A and SP-D can be important clinical markers for alveolar cell collapse or leakage, which are very common features of SARS CoV-2 infection—mediated lung injury, resulting in the release of those proteins in the circulatory system [37]. A high level of circulatory SP-A and SP-D has been reported for H1N1 infection and pulmonary fibrosis [38], and these markers can easily be measured for early assessment of severity of lung injury, which would be very important for prognosis of fatality. Thus, taking the importance of clinical facts into account, adjunctive therapy using exogenous pulmonary surfactants can be a weapon to mitigate alveolar damage and prevent progression of later-stage syndromes. Furthermore, early detection of surfactant collectins (SP-A and SP-D) in the circulatory system can be a significant clinical marker for disease prognosis in the near future.

Conclusion

After nearly 50 years of investigation, exogenous surfactant therapy or surfactant replacement therapy has recently been seen to have an effect on critical lung diseases, especially on the survival rate in neonates with ARDS. The concept and current mode of therapy is already some steps ahead of the early stages. However, adult patients with ARDS have failed to exhibit positive effects on morbidity and mortality. Why is the result so dissimilar in the case of ARDS among the adult and neonatal groups? Herein, some studies displayed negative consequences because of the surfactant's inefficacy and methods of delivery. As the worldwide severity of COVID-19 pneumonia escalates, researchers should not ignore the potential of this promising therapy and should aim to transform fundamental research on exogenous surfactants into medical application. Accurate timing of administration, the effective dose, suitable delivery methods, the selection of a particular effective drug among varied types of surfactants that show differences in density or viscosity, and the course of ventilation before and after therapy should be the prime areas of focus. Although the sensitivity of complex surfactant biochemistry is one of the major hindrances toward clinical application, pursuing deeper investigations may lead to the discovery of missing pieces in the puzzle.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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** of outstanding interest

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