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The use of aprotinin and surfactant in the treatment of viral infections, including SARS-CoV-2

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Abstract: Aprotinin is a non-specific protein inhibitor of serine proteases, while pulmonary surfactant is a complex of specific lipids, proteins, and carbohydrates, having amphiphilic properties. Both substances have an antiviral effect – aprotinin prevents proteolytic digestion of the spine, thus inhibiting the initial stage of infection. Surfactant, on the other hand, plays an important role in the first defense mechanism of the lungs, acting as a barrier. This article indicates the possibility of combining the action of aprotinin and surfactant in one drug, which may be beneficial due to the synergy of action, as well as for economic reasons for the manufacturer - both substances can be obtained from one raw material - bovine lungs.

Keywords: aprotinin, pulmonary surfactant, SARS-CoV-2, viral infections, serine protease inhibitor, BPTI

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1. APROTININ

1.1. Basic information

Aprotinin is a nonspecific protein inhibitor of serine proteases and belongs to the superfamily of bovine pancreatic trypsin inhibitors (BPTI)/Kunitz type [Fritz & Wunderer, 1985]. Protease inhibitors are commonly found in animal and plant tissues, as well as microorganisms, in various forms [Laskowski & Kato, 1980]. Their functions involve regulating protein synthesis and enzyme activity, preventing activation of protease zymogens by inhibiting proteolysis [Fioretti et al., 1982]. In bovine organs, there are four similar protease inhibitors with low molecular weights and an alkaline isoelectric point. One of these inhibitors is aprotinin. [Fioretti et al., 1982; Fioretti et al., 1987]. The presence of aprotinin and similar inhibitors has been detected in the following bovine organs: lymph nodes, pancreas, lungs, parotid glands, spleen, liver, seminal vesicles, salivary glands, ovaries, heart, thyroid, posterior pituitary, rumen mucosa, cartilage, aorta [Laskowski & Kato, 1980; Fritz & Wunderer, 1985]. Immunofluorescence technique demonstrated aprotinin's occurrence in mast cells and its activity as an intracellular

substance due to its biological function [Fritz & Wunderer, 1985; Fioretti et al., 1987]. Biochemically, aprotinin is a polypeptide with a molecular weight of 6412 and consists of 58 amino acid residues arranged in a single polypeptide chain. Aprotinin is extremely stable, it can be heated up to 100°C in an acidic environment without loss of activity. As its inactivation begins at a pH 12.8, it can be exposed to pH within the range of 1 to 12.6. Its resistance to digestion by other proteases is remarkable. The activity of aprotinin is expressed in kallikrein inactivating units (KIUs), one million of which corresponds to 140 mg of purified aprotinin [Robert et al., 1996]. Aprotinin has a wide substrate specificity and is an inhibitor of several serine proteases and their derivatives such as trypsin, anhydrotrypsin, chymotrypsin, plasmin, kallikrein, elastase, urokinase [Fritz & Wunderer, 1985]. The dose of aprotinin needed for inhibition varies for other enzymes, e.g. for plasmin it is 40 KIU/ml and for kallikrein it is 200 KIU/ml [Westaby, 1993]. 1 mg of aprotinin in drug form corresponds to 7143 KIU [Sodha et al., 2006].

1.2. Methods of obtaining aprotinin

For commercial purposes, aprotinin is mainly isolated from the bovine pancreas, bovine parotid glands, and pork lungs [Fritz & Wunderer, 1985; Xin et al., 2015]. It is estimated that the lungs contain about 1500 KIU, and the pancreas and salivary glands about 400 KIU in one gram of dry matter [Fritz & Wunderer, 1985].

Obtaining high-purity aprotinin with high efficiency is a challenging task. There are various methods available for obtaining aprotinin, but most involve using affinity chromatography in combination with other techniques such as ion exchange chromatography, hydrophobic chromatography, gel filtration, ultrafiltration, and salting. The order in which these techniques are used can vary [Fioretti et al., 1982; Siekmann et al., 1987; Ikekita et al., 1992; Dias et al., 2008; Xin et al, 2015]. Recombinant aprotinin expressed by *S. cerevisiae and* E. coli is purified by successive steps using ion chromatography, hydrophobic chromatography, affinity chromatography, and ultrafiltration [Zhong et al., 2007; Meta et al., 2009; Sun et al., 2009].

The method of choice for laboratory scale is affinity chromatography followed by acid extraction [Fritz & Wunderer, 1985]. However, when it comes to large-scale production, affinity chromatography is not very economical and inefficient due to the low stability of trypsin and kallikreins on the column, the susceptibility to binding of other inhibitors, as well as low binding efficiency [Xin et al., 2015].

1.3. Application of aprotinin

Aprotinin was introduced into clinical use in 1953 for the treatment of acute pancreatitis. It has been used in septic and hemorrhagic shock, respiratory distress syndrome, and multi-organ injuries. In the 1960s, aprotinin was first used in cardiac surgery. This led to increased research on its properties and applications, and eventually, its use for reducing blood loss during surgery [Westaby, 1993; Sodha et al., 2006].

Aprotinin was approved by the FDA in 1993 as a drug to reduce intraoperative blood loss and reduce the need for blood transfusions in patients undergoing coronary artery bypass grafting (CABG) with cardiopulmonary bypass. Its use has been extended to aortic surgery, pumpless CABG, orthopedics, neurology, and transplantation [Sodha et al., 2006].

Aprotinin can deactivate free plasmin, but it has little effect on bound plasmin. It is believed that physiologically, aprotinin is not an antifibrinolytic agent, but an improvement in hemostatic defects associated with excessive plasmin activity [Royston, 2015]. Thanks to the beneficial effects of aprotinin on hemostasis, it was possible to reduce the need for blood transfusion and decrease the resulting risks, such as transmission and infectious diseases, immunosuppression, acute transfusion lung injury, graft-versus-host reactions, and avoid anemia and its debilitating consequences [Sodha et al., 2006]. The effectiveness of aprotinin as a hemostatic agent is indisputable. It is considered more effective than other drugs such as prostacyclin, dipyridamole, desmopressin, and aminocaproic acid [Westaby, 1993].

Prophylactic use of aprotinin reduces blood loss by 40% and the need to replace blood products by 50% during cardiac, hepatic, orthopedic, and vascular surgeries [Robert et al., 1996]. The benefits of aprotinin include reduction of intra- and postoperative bleeding, shortening the duration of surgery, reducing the risk of surgical bleeding consequently lowering the need for blood from the donor, increasing neurocognitive functioning, reducing post-operative stroke [Robert et al., 1996; Sodha et al., 2006]. The risks associated with aprotinin include anaphylaxis, transient renal dysfunction in some patients, and interaction with angiotensin-converting enzyme inhibitors [Sodha et al., 2006]. The plasma half-life of aprotinin is less than 2 hours, so continuous infusion is necessary to maintain an adequate inhibition level. Aprotinin is a relatively weak immunogen, no aprotinin-specific antibodies have been detected in serum during or after treatment. Despite this, it is recommended to avoid high doses due to its high alkalinity [Westaby, 1993]. Aprotinin cannot be administered orally due to inactivation in the gastrointestinal tract [Robert et al., 1996].

1.4. Aprotinin in the treatment of viral infections

The first reports of aprotinin's ability to prevent viral infection by suppressing the digestion of ortho-myxo- and

paramyxovirus fusion proteins in mouse lungs date back to 1984 when aprotinin was administered intraperitoneally and nasally [Zhirnov et al., 1984]. The authors of these studies then extended the route of administration of aprotinin in the form of a low-molecular-weight aerosol for inhalation, proving that a small dose has a therapeutic effect on respiratory infections [Ovcharenko & Zhirnov, 1993]. The antiviral potential of aprotinin has been studied by this group of Russian researchers since at least 1982, resulting in more than 17 publications by Oleg Zhirnov [e.g. Zhirnov et al., 1982; Zhirnov et al., 1984; Ovcharenko & Zhirnov, 1993; Zhirnov et al., 1996; Zhirnov et al., 2011].

The antiviral potential of aprotinin results from the fact that enveloped viruses, such as influenza viruses, paramyxoviruses, and coronaviruses, have glycoproteins located outside the lipid bilayer of the virion in the form of a spike. This spine undergoes posttranslational proteolytic digestion, which is a prerequisite for viral infection and its spread in the host organism. Digestion of glycoproteins is carried out by trypsin-like proteinases, whose inhibitor is aprotinin [Ovcharenko & Zhirnov, 1993; Jiang et al., 2010; Kido et al., 2019].

Antiviral chemotherapeutic agents, in addition to specific drugs (such as amantadine derivatives, oseltamivir, and zanamivir), can be targeted at the level of the virus or the host. Many viruses (e.g. influenza) require activation by host proteases. Therefore, protease inhibitors such as aprotinin can be classified as host-targeted antiviral substances. The advantage of this solution is that the resistance of the virus to a given substance occurs much slower. It is thought that viral infection in the human respiratory system appears to amplify through a "vicious circle" mechanism in which epithelial cell infection stimulates host proteases, which trigger proinflammatory proteolytic cascades resulting in the activation of progeny viruses. Protease inhibitors can act in two ways, suppressing multicycle viral replication and alleviating inflammation. [Kido et al., 2007; Zhirnov et al., 2011].

It is believed that the therapeutic effectiveness of aprotinin is due to its antiviral, and anti-inflammatory activities, which can further reduce the pathology at the site of infection, and lead to a faster resolution of the disease. Anti-inflammatory activity is due to the ability to inhibit various mediators, which can extinguish inflammatory responses such as leakage of plasmin, kallikrein, and thrombin, transmigration of monocytes into lung tissue, edema, tissue oxidative stress, and impaired mucociliary clearance. These inflammatory factors are components of the pathogenesis of the influenza virus [Zhirnov et al., 2011].

Aprotinin effectively inhibited the digestion of haemagglutinin and thus inhibited the replication of the 2009 pandemic H1N1 influenza virus in many host systems, including the human respiratory epithelium [Zhirnov et al., 2011]. Russian researchers also conducted clinical trials during a seasonal outbreak of acute respiratory disease caused mainly by influenza and parainfluenza viruses. They were able to demonstrate efficacy by administering aprotinin aerosol to 84 patients [Zhirnov et al., 1996].

The Russian authors, mentioned many times, propose two modes of antiviral therapy administration using aprotinin. The first is the aerosol mode of administration to fight against upper respiratory tract infection in the nasopharyngeal and tracheobronchial areas. The second is a systematic intravenous mode of injection, based on previous indications that up to 10% of the injected dose is deposited in bronchopulmonary tissue [Zhirnov et al., 2011].

In vivo studies conducted on highly pathogenic viruses with a multibasic cleavage site (also characterizing SARS-CoV-2 [Hoffmann et al., 2020]) have shown a delay in the time of death and a reduction in viremia after intraperitoneal injection of aprotinin [Zhirnov et al, 1982].

SARS-CoV-2 infection is mediated by angiotensin-converting enzyme 2 (ACE2). The binding of the spike to ACE2 depends on the digestion of the spine at three sites by endogenous proteases, mainly transmembrane serine protease 2 (TMPRSS2), whose activity can be inhibited by serine protease inhibitors [Bojkova et al., 2020]. Local therapy of the respiratory tract and lungs with an aprotinin aerosol may be of particular importance in the early stages of COVID-19 infection when viral replication can be suppressed. In addition, aprotinin can prevent lung damage by inhibiting matrix metalloproteinases, thereby stopping the release of cytokines that result in severe systemic COVID-19 disease [Bojkova et al., 2020; Solun & Shoenfeld, 2020].

Two prospective methods of administration are being studied in clinical trials in Russia. These studies are designed to test the efficacy and safety of aprotinin in patients hospitalized with COVID-19. The observational study will be conducted in two stages. In the first stage, the efficacy and safety of intravenous infusion of aprotinin as adjuvant therapy to standard therapy will be examined. In the second stage, two groups will be considered: the first group will receive an inhaled aprotinin in addition to standard care, and the second group will receive intravenous aprotinin in combination with favipiravir [National Library of Medicine (NLM), NCT04527133].

2. SURFACTANT

2.1. Structure and functions of pulmonary surfactant

Pulmonary surfactant (PS) is a complex of specific lipids, proteins, and carbohydrates, having amphiphilic properties

[Van Golde et al., 1988]. It is synthesized and secreted by alveolar epithelial cells type II (pneumocytes). A characteristic feature of these cells is the presence of lamellar bodies, which are responsible for storing surfactant [Wright & Dobbs, 1991]. The main function of the pulmonary surfactant is related to the stabilization of the structure of the alveoli of mammals by lowering the surface tension at the air-liquid interface during the respiratory cycle. Spontaneously created stable film at the air-to-air interface prevents bubbles from collapsing and reduces respiratory effort. The different components of PS constitute the first barrier against pathogens entry to the rest of the body [Autilio & Pérez-Gil, 2019].

The main components of pulmonary surfactants are lipids (~92%), mostly phospholipids: saturated phosphatidylcholine (DPPC – dipalmitoylphosphatidylcholine) ~ 40%, unsaturated phosphatidylcholine (PC) ~ 25%, and phosphatidylglycerol (PG) ~ 10%. Cholesterol constitutes ~ 5-8%, while neutral lipids constitute 8-10% of the total mass of pulmonary surfactant [Parra & Pérez-Gil, 2015] The remaining 8% includes specific apoproteins, which can be divided into two types: hydrophobic (SP-B and SP-C) and hydrophilic (SP-A and SP-D) [Perez-Gil & Weaver, 2010; Parra & Pérez-Gil, 2015].

The surfactant's lipid component reduces surface tension at the air-liquid interface in the alveoli. The chemical structure of phosphatidylcholines, which are the main ingredient of PS, determines their function. PC and PG each have a three-carbon skeleton with a hydrophilic group (choline or glycerol) that interacts with the liquid phase, and a highly hydrophobic lipid side chain (acyl groups). The DPPC's side chain is a completely saturated palmitic acid, allowing organized monolayers to form, and providing strong compression. This allows water molecules to be excluded, and surface tension to be reduced as much as possible during exhalation with low lung volume, which is a crucial feature [Possmayer et al., 1984; Chakraborty & Kotecha, 2013]. The mechanical properties of surfactant membranes and films, and how they relate to the biological function of surfactants in the lungs, were summarized by E. Parra and J. Pérez-Gil in 2015.

Additionally, certain surfactant proteins are important for the proper activity of the pulmonary surfactant. Protein A makes up about 50% of these proteins, and consists of polypeptide chains, with collagen-like sequences at the N terminal and with C-lectin characteristics at the C terminal [Benson et al., 1985; Patthy, 1987; Haagsman et al., 1987]. A study by Korfhagen et al. (1998) shows that this protein does not play a key role in the breathing mechanism, while Casals et al. (1993) proved that it increases the interfacial adsorption of phospholipids. SP-A enhances the activity of SP-B, and SP-C proteins preserve the integrity of the packed protein-lipid complex and prevent serum components from inhibiting the surfactant [Hentschel et al., 2020]. In addition to its role in maintaining surfactant function, SP-A, along with protein D (also belonging to the protein family of collectins), plays a crucial role in innate immunity. It can bind a wide variety of microorganisms such as viruses, bacteria, and fungi, as well as allergens and environmental inorganic substrates [Ariki et al., 2012; Lawson & Reid, 2000]. The SP-D protein binds to the minor components of the surfactant – phosphatidylinositol and glucosylceramide – but its role in surfactant homeostasis remains unclear. There is a wide distribution of expression of this protein in mammalian cells, probably in line with its role as an immune defense molecule [Kishore et al., 2006**;** Hentschel et al., 2020].

Hydrophobic proteins B and C are very important for the biophysical activity of the surfactant. Their presence is essential for efficient interfacial adsorption, film stability, and surfactant redispersion in breathing cycles [Parra & Pérez-Gil, 2015; Serrano & Perez-Gil, 2006; Cruz et al., 2000]. Lack of SP-B expression impairs the direction, storage, and function of phospholipids and surfactant proteins, causing respiratory failure at birth [Clark et al., 1995]. SP-C deficiency is not so crucial for breathing and survival, but its presence prevents the development of chronic and severe respiratory pathologies [Glasser et al., 2009; Lawson et al., 2005].

2.2. Preparation and therapeutic use of pulmonary surfactant

The pulmonary surfactant occurs mainly as intracellular (present in lamellar bodies) and extracellular (released into the alveolar space). Most of our knowledge about this complex comes from extracellular surfactant studies. The intracellular surfactant is similar to a vesicular material isolated from the same animal material [Harwood, 1987].

Choosing the right method of obtaining and then purifying surfactant is extremely important and should be carefully thought out because it affects the composition of the surfactant obtained [Goerke, 1974]. Initially, pulmonary edema foam was used as a source of pulmonary surfactant [Pattle, 1955] and ground lungs [Brown, 1964], now fractionated lung homogenates and alveolar washings are more commonly used, which are then centrifuged in density gradient and purified using column chromatography [King & Clements, 1972; Hall et al., 1994]. Organic extraction using a mixture of chloroform and methanol as organic solvents is also used to obtain lung surfactant [Takahashi & Fujiwara, 1986]. This method is commonly used to isolate lipids from biological samples, enabling the extraction of a wide range of lipid forms [Bligh & Dyer, 1959]. The use of organic solvents for extraction from bronchoalveolar lavage (BAL) has been confirmed through in vitro studies, which demonstrate the possibility of reproducing surfactant properties and reducing surface tension [Curstedt et al., 1988].

The absence, deficiency, or abnormal metabolism of surfactant can result in severe respiratory distress in both newborns and adults. Some examples include neonatal acute respiratory distress syndrome (RDS) and acute respiratory distress syndrome (ARDS). Over the years, a variety of surfactants derived from animal or human sources have been developed and tested in clinical trials [Seger & Soll, 2009]. Currently, the only commercially available ones are those obtained by washing bovine lungs (like calfactant, and bovactant) and by grinding and centrifuging porcine or bovine lung tissue (e.g. poractant alpha and beractant) [Hentschel et al., 2020]. Sager and Soll [2009] conducted an analysis of thirteen randomized controlled trials that demonstrated the effectiveness of treating infants diagnosed with respiratory distress syndrome with animal surfactant extract. The results showed improved clinical outcomes, including a reduced risk of lung rupture (pneumothorax), a reduced risk of lung damage (interstitial emphysema), a reduced risk of death, and a reduced risk of chronic lung injury (broncho-pulmonary dysplasia) in these neonates.

However, natural surfactants have some limitations, such as inconsistent production (related differences in the composition and concentration of phospholipids and proteins), cost, and biological risks (potential immune risk or infectious side effects) [Hentschel et al., 2020]. Therefore, attempts were made to obtain synthetic surfactants. The first-generation protein-free surfactants had a lower effect than those of animal origin [Ardell et al., 2015], which is why functionally important proteins were added to the third-generation surfactants: Lusupultide (Venticute®) – containing recombinant SP-C and Lucinactant (Surfaxin®) – containing an analog of a peptide mimicking SP-B. Venticute®, which has been tested in adults with acute respiratory distress syndrome, despite some improvement in oxygenation, did not provide the expected therapeutic benefit [Spragg et al., 2011] and was therefore never commercialized. Lucinactant was compared with beractant and poractant alfa, with results comparable to surfactants of animal origin [Sinha et al., 2005; Moya et al., 2005], eventually leading to its FDA approval in 2012 for the prevention of respiratory distress syndrome (RDS) in premature infants at high risk for RDS. Another third-generation surfactant – CHF5633 (Chiesi Farmaceutici S.p.A., Parma, Italy), containing analogs of two human peptides, SP-B and SP-C, showed promising results in RDS therapy: rapid and sustained improvement in oxygen demand in 98% of infants, good tolerability, and no unexpected side effects [Sweet et al., 2017]. In completed phase two clinical trials, treatment with CHF5633 demonstrated similar efficacy and safety as poractant alfa in preterm infants with moderate to severe RDS [Ramanathan et al., 2020].

2.3. The role of pulmonary surfactants in viral infections

Pulmonary surfactants, in addition to lowering the surface tension in the alveoli (thus preventing them from collapsing and facilitating gas exchange), play an important role in the first defense mechanism of the lungs, since they act as a barrier, remove pathogens and modulate inflammatory responses. In particular, pulmonary collectins SP-A and SP-D bind to and are involved in the removal of various bacterial, fungal, and viral pathogens and may impair the antigen-induced immune function of effector cells [Han & Mallampalli, 2015].

As pattern recognition molecules, SP-A and SP-D proteins play a significant role in host defense, and their inhibitory effect against various pathogens has been well documented in numerous studies [LeVine et al., 2004; Hsieh et al., 2018; Qian et al., 2013]. The first in vitro studies showing that SP-D inhibits influenza A virus (IAV) was conducted by Hartshorn's group [1994]. The SP-D protein is capable of identifying and disabling various types of influenza virus subtypes. This protein binds with the influenza A virus (IAV) and helps to protect against an immediate infection by collecting viral particles, which results in a decrease in the number of infectious particles in the lungs [Hillaire et al. 2013].

Both SP-A and SP-D interact with different cells of the immune system and regulate the innate immune response in the lungs. The SP-D protein acts chemotactically on neutrophils [Hartshorn et al., 1998], while SP-A, as an immunomodulator, can inhibit the maturation of dendritic cells and inhibit the release of excess amounts of IL-8 by eosinophils [Han & Mallampalli, 2015]. Both proteins interact with CD14 and Toll-like receptors (TLRs) on the surface of alveolar macrophages [Whitsett, 2005] and may inhibit the TLRs-induced proinflammatory response [Sato et al., 2003].

Pulmonary surfactant proteins not only play a role in the lung's defense mechanism but also two minor components of phospholipids, namely phosphatidylglycerol (PG) and phosphatidylinositol (PI). These components are present in alveoli in high concentrations and regulate multiple Toll-like receptors (TLR2/1, TLR4, and TLR2/6). They exhibit anti-inflammatory effects by antagonizing TLR activators [Numata & Voelker, 2022]. These lipids also exhibit antiviral activity against many respiratory viruses, including RSV, influenza A viruses, and more recently SARS-CoV-2 [Numata & Voelker, 2022].

Respiratory illnesses such as influenza or SARS-COV-2 mainly attack type II pneumocytes. This causes a change in the production of surfactants, which leads to shortness of breath and acute respiratory distress syndrome in patients with COVID-19 [Ghati et al., 2021]. Similar to other coronaviruses, SARS-CoV-2 enters and infects type II

follicular cells using angiotensin-converting enzyme receptor 2 (ACE2) [Hoffmann et al., 2020; Lan et al., 2020]. Damage to these cells causes a drastic reduction in the production and secretion of surfactant into the follicular space [Mirastschijski et al., 2020].

During the COVID-19 pandemic, the surfactant attracted attention, especially for its defensive and immuno-modulatory properties of proteins and lipids [Han & Mallampalli, 2015]. The use of exogenous surfactants has shown great promise in treating respiratory distress syndrome in infants [Zhang et al., 2015]. Additionally, it may aid in the healing of damaged alveolar cells and prevent respiratory failure [Rahaman et al., 2021]. A surfactant can restore immune homeostasis because it is a strong defender against the virus itself [Takano, 2020]. As such, some researchers have proposed using surfactant therapy to treat COVID-19 patients [Takano, 2020; Mirastschijski et al., 2020; Ghati et al., 2021; Cattel et al., 2021].

Reduction of surface tension in the alveoli after administration of a surfactant improves gas exchange, which increases oxygenation, and thanks to early administration, shortens the time of mechanical ventilation, improves lung function, and improves the recovery of patients [Mirastschijski et al., 2020; Cattel et al., 2021]. To achieve a synergistic effect, exogenous surfactants can be combined with anti-inflammatory, antioxidant, antiviral, and antibacterial agents such as Ambroxol [Kumar 2020].

Currently, several clinical trials using exogenous pulmonary surfactants in patients with ARDS [https://clinicaltrials.gov] are registered. The "Surfactant-BL in Adult Acute Respiratory Distress Syndrome Due to COVID-19" study conducted in Russia is an open-label study to evaluate the efficacy and safety of the inhaled approved drug Surfactant-BL (Biosurf LLC, Russia) as part of the combined therapy of acute respiratory distress syndrome (ARDS) in patients with SARS-CoV-2 coronavirus infection (COVID-19). The primary measure of outcome is the mean duration of oxygen therapy in the treatment group and the control group [National Library of Medicine (NLM), NCT04568018]. In France, a clinical trial called "Curosurf® in Adult Acute Respiratory Distress Syndrome Due to COVID-19 (Caards-1)" is underway. This is a randomized, controlled phase II study of Poractant Alfa (Curosurf®) by bronchial administration under the guidance of fiber optic bronchoscopy in acute respiratory distress syndrome (ARDS) caused by COVID-19 viral pneumonia [National Library of Medicine (NLM), NCT04384731]. The clinical trial "Exogenous Surfactant Through Nebulizer Mask on Clinical Outcomes in Covid-19 Patients (CovidSurf)" aims to assess the effect of nebulized surfactant administered through a nebulizer face mask on clinical outcomes in Covid-19 patients [National Library of Medicine (NLM), NCT04847375]. Patients in the completed study "The Safety and Preliminary Tolerability of Lyophilized Lucinactant in Adults With Coronavirus Disease 2019 (COVID-19)" received the synthetic surfactant lucinactant (KL4-surfactant). Multicenter, single treatment was designed to assess the safety and tolerability of lyophilized lucinactitant in adults with acute lung injury associated with COVID-19 [[National Library of Medicine (NLM), /NCT04389671].

3. THE COMBINATION OF APROTININ AND SURFACTANT IN THE TREATMENT OF VIRAL INFECTIONS

This publication describes the antiviral potential of aprotinin and surfactant. The synergy of action of both substances may probably consist in the fact that both are host-targeted antiviral substances, but both act in a different way and target a different place in the viral infection process. Aprotinin inhibits virus activation by host enzymes, while surfactant acts as a first line of defense by removing viruses from the lungs. Additionally, both substances have a more general effect. Aprotinin has anti-inflammatory properties by inhibiting various infection mediators, while surfactant reduces tension in the alveoli and improves lung function and gas exchange. Moreover, surfactant therapy could be complemented by aprotinin's anti-inflammatory properties to reduce surfactant dysfunction caused by inflammation. This is particularly important in conditions such as acute respiratory distress syndrome (ARDS), which may be caused by a virus infection (e.g. SARS-CoV-2 virus), where inflammation can disrupt surfactant production and function through various mechanisms, leading to impaired gas exchange, lung damage, and respiratory failure [Suter, 2006]. Aprotinin's ability to reduce inflammation could enhance surfactant therapy by addressing inflammatory processes that compromise lung function. Furthermore, aprotinin protects surfactant proteins and lipids from degradation by inhibiting certain proteases like elastase and plasmin. This preservation can enhance the effectiveness of surfactants in reducing surface tension and maintaining lung function. All of these effects contribute to the antiviral synergy that comes with combining both substances in a single drug.

The Polish patent [Czarnecki et al., 2012] describes a proposal to combine aprotinin and surfactant to create a drug that can prevent infection from influenza viruses. According to in vivo experiments conducted by the patent developers, the combination of aprotinin and surfactant was found to be effective for eight hours at a dosage four times lower than that of aprotinin alone, with the latter only being effective for two hours. These findings demonstrate that the combination of the two substances exhibits a clear synergy effect, which has proven to be particularly effective in prophylactic protection of mice against influenza infection. The use of both substances represents a new approach to the prophylactic prevention of viral infections. [Czarnecki et al., 2012].

Technologically speaking, the combination of aprotinin and surfactant is an economically beneficial solution. Both substances can be obtained from a single source, which is bovine lungs. After extracting surfactant from the meat tissue through an organic extraction process, the remaining tissue can be used for the production of aprotinin in an aqueous extraction process. This method aligns with the zero-waste philosophy.

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