

A Narrative Review of a Pulmonary Aerosolized Formulation or a Nasal Drop Using Sera Containing Neutralizing Antibodies Collected from COVID-19–Recovered Patients as a Probable Therapy for COVID-19

Nishat Fatima, MPharm, PhD;  Vichitra Kaushik, MPharm, PhD; Amjad Ayoub, BPharm, MSc 

School of Pharmacy, Al-Hawash Private University, Homs, Syria

Correspondence:

Amjad Ayoub, BPharm/MSc;
School of Pharmacy, Al-Hawash Private University, P.O. Box: 22743/99, Homs, Syria

Tel: +963 31 2080

Fax: +963 31 7747935

Email: amjad.ayoub@hpu.edu.sy

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Abstract

Coronavirus disease 2019 (COVID-19) emerged as a new contagion during December 2019, since which time it has triggered a rampant spike in fatality rates worldwide due to insufficient medical treatments and a lack of counteragents and prompted the World Health Organization to declare COVID-19 a public health emergency. It is, therefore, vital to accelerate the screening of new molecules or vaccines to win the battle against this pandemic. Experiences from previous epidemiological data on coronaviruses guide investigators in designing and exploring new compounds for a safe and cost-effective treatment. Several reports on the severe acute respiratory syndrome (SARS) epidemic indicate that severe acute respiratory syndrome coronavirus (SARS-CoV) and the novel COVID-19 use angiotensin-converting enzyme 2 (ACE2) as a receptor for binding to the host cell in the lung epithelia through the spike protein on their virion surface. ACE2 is a mono-carboxypeptidase best known for cleaving major peptides and substrates. Its degree in human airway epithelia positively correlates with coronavirus infection. The treatment approach can be the neutralization of the virus entering lung epithelial cells by using sera containing antibodies collected from COVID-19–recovered patients. Hence, we herein propose a pulmonary aerosolized formulation or a nasal drop using sera, which contain antibodies to prevent, treat, or immunize against COVID-19 infection.

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Keywords • COVID-19 • Angiotensin-converting enzyme 2 • Serum • Neutralizing • Antibodies

What's Known

- The systemic-route administration of sera containing neutralizing antibodies collected from COVID-19–recovered patients as a probable therapy for COVID-19 is already known.

What's New

- We tried a pulmonary aerosolized formulation or nasal drop using sera containing neutralizing antibodies collected from COVID-19–recovered patients as a probable therapy for COVID-19.

Introduction

Pneumonia related to severe acute respiratory syndrome (SARS), which first appeared in Wuhan, China, in December 2019, was termed “coronavirus disease 2019 (COVID-19)” or “coronavirus 2 (SARS-CoV-2)” by the World Health Organization (WHO).¹⁻³ Within three months of its rapid global spread, the contagion was declared a pandemic by the WHO on March 11, 2020.³ The earlier published reports regarding coronaviruses showed that

they evolve strains that can infect, in addition to animals, humans. Coronaviruses have been categorized into four genera: alpha-CoV, beta-CoV, gamma-CoV, and delta-CoV.⁴ Studies have revealed that beta-CoV has had three zoonotic outbreaks. Severe acute respiratory syndrome coronavirus (SARS-CoV), which affected around 8000 people between 2000 and 2003,^{5, 6} is considered a lineage B beta-CoV originating from bats and palm civets.⁷ In the year 2012, Middle East respiratory syndrome coronavirus (MERS-CoV), a lineage C beta-CoV, was identified as the causative agent of a severe respiratory syndrome in Saudi Arabia.⁸ A novel coronavirus commonly known as “SARS-CoV-2” or “COVID-19” became a pandemic that has largely affected people around the globe and caused severe pneumonia since December 2019.^{9, 10} The most common signs of COVID-19 complications are fatigue, myalgia, and mild-to-severe respiratory distress causing coughs and difficulty in breathing.⁴

SARS-CoV and COVID-19 viruses bind with the angiotensin-converting enzyme 2 (ACE2) receptor to enter the cell through the spike (S) protein on their virion surface, setting off the fusion of the viral membrane and cell membrane.⁷ ACE2 is highly expressed in the lungs, and pulmonary ACE2 appears to be involved in maintaining a balance between circulating angiotensin II/angiotensin I–VII levels. Pulmonary vasoconstriction induced by angiotensin II in response to hypoxia is vital in treating patients with pneumonia or lung injury. ACE2 is a mono-carboxypeptidase known for cleaving major peptides and substrates.¹¹ The S protein is responsible for syncytial development between infected and noninfected receptor cells, indicating that the function of the S protein is not limited to the virion state alone.¹² The immunogenicity of the S protein sequence in coronaviruses may be still under study, but this protein is known to serve as a common binding site to SARS, MERS, and COVID-19.¹³

It would be worthwhile to produce a novel therapeutic formulation capable of not only curbing the spread of the 2019 novel coronavirus (2019-nCoV) but also treating acute respiratory distress syndrome (ARDS). Indeed, in the current absence of effective COVID-19 vaccines and antiviral therapies, various pharmaceutical and biotechnology companies are seeking a solution. A vaccine rollout, however, follows lengthy processes of development and testing. Several antiviral medicines are undergoing trials, and the initial results are expected shortly.

Individuals with weak immune systems are naturally more at risk of developing COVID-19

related complications than those with strong immune systems. The enhancement of the immune response to this virus and the prevention or treatment of COVID-19 may require immunotherapy with immunoglobulin G (IgG).^{14, 15} There are two functional portions of IgG antibodies: the F(ab₀)₂ fragment, which is responsible for antigen recognition, and the crystallizable fragment (Fc), which is essential for immune response activation by interacting with the Fcγ receptors of B lymphocytes and other innate immune cells. The Fc fragment also plays an important role in complement activation and microorganism clearance.¹⁶

In general, sera produce anti-coronavirus antibodies from almost all healthy adults.¹⁷ Research has shown that human coronavirus NL63 (HCoV-NL63) infection could be restrained with intravenous IgG obtained from the sera of healthy adults.^{13, 15} Boukhvalova and others showed that IgG obtained from donors with high-titer antibodies was more effective against the respiratory syncytial virus in immunocompromised patients than commercially available polyclonal therapeutic IgG products.¹⁴ The mechanism is two-pronged: viral replication control and damage limitation in the lung parenchyma and epithelial lining.

The treatment approach can be the neutralization of the virus entering cells, which mirrors the action performed by antibodies. Sera containing antibodies collected from COVID-19–recovered patients could be used to treat or prevent COVID-19 and to build up immunity against it.¹⁶ To that end, in the present study, we propose a novel pulmonary aerosolized formulation or a nasal drop for the administration of sera containing antibodies collected from COVID-19–recovered patients as a probable therapy for or immunization against COVID-19.

Genetic Structure of the Coronavirus

The full-genome sequencing of viral RNA has shown that the virus that caused COVID-19 is phylogenetically related to the first SARS coronaviruses isolated from Chinese horseshoe bats between 2015 and 2017.^{17, 18} These viruses are enveloped and possess a positive single-stranded RNA genome of approximately 27 to 32 kilobase (kb). The genome encodes a polyprotein that is present at 5' terminal, at two-thirds of the genome and comprises all proteins essential for the replication of RNA. Virus entry relies on the binding of the surface unit, S1, of the S protein to a cellular receptor, which provides a linkage between the virus and the target cell surface. The S protein cleavage at S1/S2 and more specifically the S2 subunit causes the

fusion of the membranes of the virus and the host cell.¹⁹ SARS-S deploys ACE2 and uses cellular serine protease TMPRSS2 for the priming of the S protein.²⁰⁻²² The interaction between SARS-S and ACE2 has been illustrated microscopically, and the presence of ACE2 determines the capacity of the transmission of the SARS-CoV infection.^{23, 24} The rate of amino acid similarity between SARS-S and SARS-2-S is 76%.²⁵

Tang and others showed two primary lineages for COVID-19: L and S types. The S type was originated first, and the L type, which was predominant, evolved from the former during the early weeks of the outbreak. The older S type appeared to be milder and much less infectious than the L type. Tang and colleagues posited that these two had different transmission rates: The L type spreads quickly and is currently more aggressive in nature than the S type. The S and L types can be defined clearly by just two strongly connected single-nucleotide polymorphisms (SNPs) at positions 8,782 (orf1ab: T8517C, synonymous) and 28,144 (ORF8: C251T, S84L). Analysts at the College of Glasgow contended that among the study's numerous specialized issues was a crucial one: The conclusions had failed to test whether the overabundance of the L type might have happened without changes within the infectiousness of the virus.^{5, 10}

Lu and colleagues reported that physiologically, the full-length ACE2 operates in transmembrane and extracellular domain forms.¹² The extracellular domain denotes the binding of SARS-CoV²⁶ and new SARS-CoV-2¹⁵ through their S protein.²⁷

Neutralizing the Activity of Serum Antibodies

A traditional adaptive immunotherapy modality, known as "convalescent plasma treatment", has been applied for more than a century in the prevention and care of many infectious diseases with similar virological and clinical features, such as SARS MERS, MERS, and COVID-19.²⁸ Several studies have reported the sufficient efficacy and safety of convalescent plasma therapy in the treatment of the coronavirus and H1N1 in the 2009 pandemic.²⁹⁻³² In SARS, viremia develops during the first week of infection,⁵ and patients typically develop an immune response within the next week, which is more likely to cause a lethal cytokine storm.^{5, 7} Evidence also indicates that convalescent plasma therapy might attenuate the response of serum cytokine release. Thus, it is plausible to argue that convalescent plasma therapy could be a COVID-19 rescue treatment option.³³ A useful donor source of convalescent plasma may be

patients who have recovered from COVID-19 with a high neutralizing antibody titer.

Mechanism of Action of Serum Antibodies

The Fc fragment recognizes target antigens that engage immune cell effectors such as natural killer cells, granulocytes, monocytes, and macrophages upon binding to Fcγ receptors. This activation results in phagocytosis and antibody-dependent cellular cytotoxicity. On the other hand, non-neutralizing antibodies that bind to the pathogen but do not affect its replicating skill, can be used for prophylaxis and/or recovery boost.^{34, 35} It is important to note that the administration of passive antibodies as a treatment strategy can confer immediate immunity to infected patients and vulnerable individuals as well. This remarkable effect of antibodies has come into view as an immediate treatment for patients with COVID-19.

Maneuvering Serum Antibody Therapy against COVID-19 Infection

Marano and others demonstrated that convalescent plasma collected from COVID-19-recovered patients was composed of high levels of neutralizing antibodies that counteracted SARS-CoV-2 and eliminated the pathogen from blood circulation and pulmonary tissues.³⁶ It was also observed that all patients after convalescent plasma transfusion showed serum SARS-CoV-2 RNA negativity along with augmented oxygen saturation, increased lymphocyte counts, improved liver function, and reduced C-reactive protein. Their study showed that antibodies contained in convalescent plasma ameliorated the inflammation and overreaction of the immune system. The case fatality rate was 0% (0/10) and was comparable with that of SARS, which varied from 0% (0/10) to 12.5% (10/80) in four non-comparative studies using convalescent plasma treatment.³⁷⁻⁴⁰ Such observations suggest convalescent plasma therapy as a rescue option for patients with severe COVID-19. Research on SARS demonstrated a specific IgG that began to increase around week three after the onset and peaked at week 12.⁴⁰ In contrast, in another study on influenza, convalescent plasma collected from recovered patients at week 12 showed reduced mortality with a neutralizing antibody titer of 1:160 or greater.³⁰ Thus, a minimum convalescent plasma level of 1:160 is expected to be more effective after the onset of infection. These findings can form a cornerstone for trials using sera containing antibodies as therapy for patients with COVID-19.

For the treatment of 10 severe cases of COVID-19, Maillet and colleagues administered

200 mL (unit dose) of convalescent plasma collected from recovered patients as supportive care along with an antiviral agent and reported a neutralizing antibody titer of above 1:640. This convalescent plasma dose was well tolerated, and it conferred recovery from viremia in seven days. Furthermore, within three days, there was an improvement in clinical signs and paraclinical conditions, and radiological examinations showed amelioration in lung lesions within seven days.⁴¹

Duan and others reported a remarkable clinical improvement via convalescent plasma transfusion in 10 patients with extreme COVID-19.⁴² Despite a few limitations, this conventional treatment provided an insight into the treatment for patients with serious and basic COVID-19.⁴² Duan and colleagues sought to inactivate the infection with a view to diminishing a few coagulation components, particularly fibrinogen and factor VIII, in healing plasma.⁴² Nevertheless, Shen and others concluded that healing plasma, when transfused instantly without inactivating the infection, could exert a positive impact on patients with serious and basic COVID-19.⁴³

Furthermore, what is the most advantageous time to collect convalescent plasma from the donor? As was stated in the second trial edition of *Clinical Treatment of Convalescent Plasma for COVID-19*, published by the National Health Commission of China, the right time to collect donor blood is three weeks following the onset of illness.⁴⁴ Similarly, Duan and others collected donor blood three weeks after the onset of illness and four days after recovery.⁴² In contrast, in the study by Shen and colleagues, blood was collected 10 days after recovery.⁴³ Indubitably, the total antibody dose (the transfused volume of convalescent plasma multiplied by the SARS-CoV-2 neutralizing antibody titer) for adults needs further investigation.

In the study by Duan and colleagues, 200 mL of convalescent plasma with a neutralization titer of higher than 640 was transfused,⁴² whereas 400 mL of convalescent plasma with a neutralization titer of higher than 1000 was transfused in the study by Shen and others.⁴³ Nonetheless, positive clinical enhancements were confirmed in both cases. Limited availability is the most daunting hurdle in the utilization of convalescent plasma, hence the need for conclusive evidence as to what constitutes its exact maximum dosage. The issue is further compounded by the absence of data on the severity of COVID-19 infection in donors, since the active plasma of donors with varying degrees of COVID-19 severity may have varying therapeutic effects. In an investigation on 10 patients with the infection, all symptoms, especially fever, cough, shortness

of breath, and chest pain, either disappeared or exhibited improvements within one to three days during the transfusion of convalescent plasma.⁴⁵

All patients with severe COVID-19, who were registered in a study achieved their primary and secondary outcomes. A single dose of 200 mL was endured well. Additionally, an increase in oxyhemoglobin saturation was concurrent with noticeable improvements in the clinical symptoms within three days, resulting in the drastic neutralization of the viremia convalescent plasma obtained from patients, who had recovered from COVID-19 and had high levels of neutralizing antibodies capable of counteracting SARS-CoV-2 and destroying the disease-causing virus from the bloodstream and lung tissue. All the examined patients, according to that study, achieved negativity in serum SARS-CoV-2 RNA after convalescent plasma transfusion. This improvement was accompanied by elevated oxygen saturation, lymphocyte counts, enhanced liver function, and C-reactive protein. Overall, the results suggested that the inflammation and overreaction of the immune system were subdued by the antibodies contained in convalescent plasma, whereas there were insignificant improvements in those treated with convalescent plasma transfusion 14 days after the onset of illness. No adverse effects were reported by that investigation.³⁶ It is well worth reiterating the need to determine the optimal transfusion time point in view of the uncertainty in the potency of the viremia of SARS-CoV-2.

The transmission of potential pathogens is one of the dangers of plasma transfusion. As a method superior to the ultraviolet C light method, methylene blue photochemistry was applied in this study to inactivate the potential residual virus in neutralizing antibodies as much as possible.^{42, 46}

The well-being of benefactors is indubitable of paramount importance. An extra advantage on the donor's side is that many laboratory hemostatic abnormalities are observed in patients who are seriously ill with COVID-19 infection. This is viewed as a significant clinical issue taking into account the high rate of thrombotic occurrences in a population, some of whose outcomes might be kidney failure and fatalities. Repeated targeted plasmapheresis could lower and normalize the state of hypercoagulability in COVID-19-positive individuals. In countries endowed with modern portable apheresis technologies, the plasmapheresis collection process and plasma exchange are acknowledged clinical methods. For individuals unable to receive a

suitable vaccine against the COVID-19 virus, the transfusion of plasma or its stem products containing immunoglobulins from patients who have fully recovered from COVID-19, would be an extended benefit, not least in the current era when modern technologies offer the early detectability of infection. With respect to therapeutic treatments, the appropriate selection of the right product for the right patient at the right time and in the right condition can enhance transfusion precision with added benefits for both donor and recipient. Plasmapheresis with citrated anticoagulants could also provide remarkable assistance by lowering existing or COVID-19-induced hypercoagulability, as this patient population is at a greater risk for thrombosis and venous thromboembolism.⁴⁷ The caveat to be factored in is that not only do the safety and potency of convalescent plasma as a treatment for patients with COVID-19 have yet to be approved but also both donor and recipient must be fully informed about the outcome within the sphere of ethical and legal regulations.

Additionally, COVID-19 plasma should be utilized within the setting of organized research with a well-thought-out outline to decide its security and viability in comparison with the standard of care or other restorative intercessions. Indeed, in the case of experimental use, it is imperative to check the clinical and research facility pointers of security and adequacy to optimize/maximize the knowledge that may be advantageous.⁴⁸

In this respect, the use of neutralizing anti-SARS-CoV-2 immunoglobulins in COVID-19 healing plasma as such or after segregating its more enhanced immunoglobulin parcel is a curiously helpful approach, acting on the same principle of the well-established concept of inactive immunotherapy. Donors' recuperation from COVID-19 must be confirmed via pre-screening and pre-donation testing. For instance, physical examinations of such contributors are required to corroborate the nonattendance of fever and respiratory side effects. If plasma is collected earlier than 28 days after full recuperation from sickness, two non-reactive nucleic corrosive tests for SARS-CoV-2 on nasopharyngeal swabs should be performed at a minimum interim of 24 hours. The viral dormancy of recovering plasma also needs to be tested for transfusion-transmissible infections. The precise date of COVID-19 contamination, history of indications, medications taken, and date of the determination of all indications ought to be recorded and traceable. The neutralizing titers of anti-SARS-CoV-2 antibodies in totality are determined as a part of product characterization

before use. Additionally, donor blood/serum/plasma samples should be stored at -80°C for reconsidering testing and further scientific investigations. The plasma bonding of at least two donors might be useful to ensure the delivery viability of different antibodies. Considering the current paucity of published reports on convalescent COVID-19 plasma transfusion, an initial dose of 200 mL can be followed by one or two extra doses of the same quantity according to the severity of the disease and the viability of infusions. Sufficient information is also vitally important with respect to the blood/serum/plasma samples of recipients before and after transfusion.⁴⁹

Another profoundly significant issue is plasmapheresis in cases of hematological malignancies in immunocompromised patients. The elderly and individuals with underlying comorbidities have the highest morbidity and mortality rates associated with COVID-19; robust results are needed to suggest the best therapeutic interventions for the categories most vulnerable to this contagion.

COVID-19-related pneumonia is allied to the hyper-enactment of effector T cells and the intemperate generation of incendiary cytokines such as interleukin-6, interleukin-1, interferon-gamma, and tumor necrosis factor. This provocation may lead to plasma spillage, vascular porousness, and dispersed intravascular coagulation. This response, called "cytokine storm", could be a life-threatening complication of COVID-19. The immunocompromised status related to hematological malignancies may upgrade the hazard of bacterial sepsis as well as COVID-19 and other viral contaminations.⁴³ In the light of these findings, the preventive or the therapeutic use of convalescent plasma may lessen the impact of COVID-19. Nevertheless, this issue requires clarification through well-planned clinical preliminaries. The utilization of healing blood items to accomplish inactive resistance is not a novel idea, and it was proposed by the WHO as an early alternative for treating patients with Ebola infection.⁵⁰

Advantages of Pulmonary Drug Delivery

The pulmonary delivery of aerosolized drugs represents a convenient way of self-administration. Dispatching drugs to the lungs by pulmonary route is a noninvasive method. This drug delivery system is capable of skipping hepatic first-pass metabolism and reducing the possibility of drug-induced toxicity or adverse drug reactions. The alveolar area is anatomically filled with an active surface layer of phospholipids (mainly phosphatidylcholines

and phosphatidylglycerols) and several primary apoproteins. Such agents also play a pivotal role in alveolar fluid homeostasis and many mechanisms of defense. There is a viscoelastic, gel-like mucus layer of about 0.5 to 5.0 mm thickness lining the upper airways to the terminal bronchioles. The mucus layer consists of a low viscous fluid layer below and a more viscous layer on top, which surrounds the cilia (the periciliary fluid layer). This mucus layer, a mixture of glycoproteins produced by the goblet cells and local glands, forms a protective layer and eliminates inhaled particles from the airways by mucociliary transport depending on viscosity and elasticity.⁵¹

Targeted drug delivery to the lung tissue is difficult as it is highly vascularized and has an increased absorption rate for particularly lipophilic and low-molecular-weight drugs. Similarly, on reaching the alveoli, most peptides and proteins are either degraded by proteases or removed by alveolar macrophages. Here, the unique nature of the mucociliary layer acts as a barrier and contains high concentrations of protease inhibitors, which can confer protection against the degradation of peptides and proteins.⁵²⁻⁵⁵ Targeted pulmonary drug delivery, therefore, could be efficacious in the treatment of various conditions that cause injury to the lungs by deep inhalation and drug deposition.⁵¹ Aerosolized biopharmaceuticals have been used for the treatment of cystic fibrosis and asthma,^{16, 56} and limited data are available on larger complex proteins such as antibodies as aerosols.⁵⁷⁻⁶⁰ The aerosolization system is preferred for targeting aerosol deposition in the nasal cavity,⁶¹ nasal sinuses,⁶² and the respiratory tract.⁶³ Micron-sized aerosol droplets have tremendous effects on complex proteins such as monoclonal antibodies (mAbs). These proteins can aggregate or unfold during aerosol formation.⁶⁴⁻⁶⁸ To overcome aggregation at the air-liquid interface, investigators have drawn upon such surfactants as polysorbates in the formulation of mAbs.^{69, 70} This group of proteins can aggregate or grow during the formation of aerosols. In these circumstances, for the aerosolization of liquid drugs, nebulizers are convenient devices, because they provide a more moderate continuous rate of flow and a constant aerosol size distribution in the optimal range for pulmonary or nasal delivery.⁷¹ Nebulizer formulations can reduce protein aggregation and bioactivity loss.^{69, 72} Protein stability can be improved by a suitable aerosol drug formulation.⁷³ Aerosol formulations also show a positive effect on mucosal protein permeation and bioavailability.⁷⁴⁻⁷⁶ A lead inhibitor

compound, 13b, administered by inhalation route in mice inhibits the enzymes responsible for viral replication after entering the human cell. This compound is well tolerated without any adverse effects, indicating that direct administration into the lungs could be beneficial for the development of the pulmonary delivery of drugs against the coronavirus.⁷⁷ Likewise, a recently published prospective study conducted at Taihe Hospital in Wuhan City in mid-January 2020 demonstrated the efficacy and safety of recombinant human interferon alpha-1b administered as nasal drops to healthy medical staff to ensure a protective measure against COVID-19 infection.⁷⁸

Generally, low concentrations of mAbs reach the lungs after systemic administration in humans. In the treatment of lung diseases, the inhalation route is well-established for the local delivery of mAbs.⁷⁹ Neutralizing mAbs administered by intranasal or aerosol route can significantly decrease the amount of mAb required for protection against infection by one log in comparison with intraperitoneal or intravenous route. Aerosolized drug delivery is the route of choice for treating pulmonary infections.^{80, 81} The systemic administration of mAbs may also be associated with severe toxicity and adverse effects.⁸⁰

Aerosol Therapy in Sub-Intensive Patients with COVID-19

Patients with hypoxemic respiratory failure are treated with the high-flow nasal cannula (HFNC).⁸²⁻⁸⁴ The utilization of the HFNC, taking into account the scarcity of ventilators, may be a great choice sometimes in patients with asthma and chronic obstructive pulmonary disease creating severe hypoxemic respiratory failure. Here, there is a genuine concern regarding the use of the HFNC as the emission discharged from patients with COVID-19 may be inhaled by those administering it. Since the HFNC does not have a closed circuit like ventilators, there is a danger of the scattering of aerosolized infection. The careful use of the HFNC in patients suffering from COVID-19 as well as the risk/benefit ratio of aerosol drug delivery through the HFNC has yet to be fully investigated, although previous studies have shown that there is a low risk of airborne transmission with the HFNC when good interface fitting is achieved.⁸⁵⁻⁸⁷ Past investigations have reported that expanding the stream diminishes the fugitive emission and particle size amid treatment. Clinicians should use surgical masks on the faces of infected patients, if aerosolized drugs have to be administered through the HFNC and should do it in a low-pressure room.⁸⁸⁻⁹⁰ Respiratory

advisors are recommended to wear individual defensive gear, N95 mask respirators, goggles/face shields, double gloves, outfits, or cook's garment if the outfit is not liquid safe.

Aerosol Therapy in Intensive Patients with COVID-19

According to a recent study, 66% of patients with COVID-19 develop severe respiratory disorders.⁹¹ Patients with COVID-19 take nine to ten days to deteriorate and require intensive care for respiratory support.⁹² Critically ill patients with COVID-19 may need nebulizers while receiving ventilatory support. Additionally, it is important to maintain the circuit intact and prevent the transmission of the virus. It is not appropriate to deliver aerosolized medications via the jet nebulizer or the pressurized metered-dose inhaler (pMDI) because of the breakage of the circuits for the placement of the device on the ventilator circuit before aerosol therapy. Latest Chinese guidelines advocate the use of mesh nebulizers in critically ill patients with COVID-19, who are receiving ventilator support.⁸⁸ Adding medications will be easier without breaking the ventilator circuit for aerosol drug delivery as mesh nebulizers with a reservoir design can stay in-line for up to 28 days. The medication reservoir of mesh nebulizers, in contrast to jet nebulizers, is isolated from the breathing circuit, which removes the nebulization of contaminated fluids. For the improvement of the efficiency of the treatment and the further reduction of retrograde contamination from the patient, the mesh or jet nebulizer can be placed before the humidifier.⁹³⁻⁹⁸ Nebulizers can deliver a variety of drug formulations that may be needed for patients with COVID-19, which inhalers cannot. For the delivery of aerosolized medications, traditional jet nebulizers are commonly used, albeit they spew two-thirds of the emitted aerosol into the surrounding environment.⁹⁹⁻¹⁰¹

Detailed Pharmaceutical Aspects of Aerosolized Formulation

It is well established that aerosolization is one of the most important routes for administering therapeutic macromolecules to the lungs. For the efficient delivery of macromolecules, particle-based carriers have been formulated with suitable physicochemical properties that allow target-specific and controlled release. Targeting can be passive or active depending on the physiological features of the targeted environment or the constituents of the cell surface, respectively.

Particles in the alveolar barrier are either absorbed by receptor-mediated transcytosis,

paracellular passive transport, and endocytosis or engulfed by macrophages. The controlling factors for macrophage uptake are their size and molecular weight. Maximum molecular weight of 25 kDa is cleared quickly, whereas a minimum molecular weight of 40 kDa is cleared slowly. Nevertheless, for the phagocytosis process, the optimal particle size was found to be between 1.5 and 3 μm . The breakdown of the macrophage clearance mechanism can be averted by noting the fact that drug particles in delivery systems must have a size that is outside the range recognized by macrophages. The lung surfactant may induce aggregation and can promote macrophage clearance for macromolecules such as peptides, proteins, and small interfering RNAs (siRNAs)/microRNAs (miRNAs). Additionally, secretions from macrophages such as peroxidases can cause the degradation of macromolecules and lead to local immune responses.^{102, 103} Tuberculosis, lung cancer, emphysema, pneumonia, ARDS, and pulmonary edema are various disease conditions affecting the alveoli. The successful macromolecule delivery depends widely on the pathophysiology of the respiratory tract and the severity of the disease.

Macromolecules comprise a heterogeneous group of proteins, which constitute peptides (20–30 amino acid residues, also called “oligopeptides”, “cytokines”, “enzymes”, “vaccines”, “mAbs”, and “clotting factors”) and genetic material (DNAs, plasmid DNAs [pDNAs], RNAs, siRNAs, miRNAs, ribozymes, and aptamers). These molecules possess certain characteristic features such as potency and specificity due to highly selective receptor binding, decreasing the chance of off-target side effects, and making them useful for therapy. The use of macromolecules is, however, associated with some limitations. Due to their large size, hydrophilicity, molecular weight, structural instability, and low absorption at the site of entry, injections have been preferred for their delivery. Additionally, the short circulatory half-life poses another drawback with macromolecules use, which necessitates parenteral administration and decreases patient compliance.¹⁰⁴ On the other hand, aerosolized macromolecule delivery shows the potential to be deemed an important alternative method of delivery. The potential benefits of aerosolized formulation include its noninvasiveness, convenience of self-administration, and avoidance of hepatic metabolism. This route enhances the bioavailability of the bioactive compound due to the fast onset of action and requires small doses, thereby decreasing the risk of adverse reactions.¹⁰⁵ The benefits notwithstanding,

aerosolized macromolecule inhalation has its own disadvantages in its formulation, storage, and delivery.¹⁰⁶

The production and separation techniques of macromolecules are expensive. The traditional methods employed for the purification of animal tissue protein extracts result in diminished immunogenicity and specificity. To counteract these difficulties, investigators have implemented newer recombinant macromolecule production lines to increase the quantity and quality of the macromolecules and to reduce the cost. Currently, specialists are concentrating on transfected human cell lines, despite their cost, instead of *Escherichia coli* clones to have macromolecules with more strong structural resemblance to human proteins.¹⁰⁷

Macromolecules exhibit unique structural features, and their activity depends closely on their structural stability, rendering these formulations challenging. A number of dosage forms namely dry powders, aqueous solutions, liquid solutions, or suspensions in a propellant vehicle have been utilized to preserve the structural integrity of aerosol formulations. Carrier-based formulations can be used to prevent the exposure of macromolecules to enzymatic degradation and macrophage uptake by their encapsulation. For the improvement of the bioavailability of formulations, various pharmaceutical ingredients such as stabilizers, absorption enhancers, mucoadhesive adjuvants (e.g., fatty acids), surfactants, and protease inhibitors have been employed.¹⁰⁸⁻¹¹⁰ Formulation strategies have also been posited to prolong protein release (hydrogels, liposomes, micro/nanoparticles, and micelles) and action duration.¹⁰⁹ Furthermore, the selection of the suitable aerosol delivery device is governed by the type of macromolecule utilized and the status of the pulmonary target site. The other vital parameters to be considered for the evaluation of macromolecules include their toxicity, local and systemic side effects, efficacy, shelf life, and immunogenicity. There might be a risk of immunogenic reactions with macromolecules, leading to the release of antibodies as their key response.¹¹¹ Usually, the macromolecule is recognized as a foreign substance that is incorporated, refined, and presented by antigen-presenting cells, resulting in CD4 T-cell responses and elevations in the antibody titer. This type of immunogenic response can be utilized and targeted as in vaccine prophylaxis and therapeutics.¹¹² The efficient delivery of therapeutic macromolecules to the lungs through aerosolization requires a thorough understanding of macromolecules'

physicochemical characteristics, formulation type, and carrier methods adopted. Additionally, the choice of inhaler device forms the most important aspect of pulmonary delivery and depends on the pathophysiological condition of the lungs. The selection of the inhaler device is made based on patient condition, as well as formulation. Albeit well-established marketed devices, nebulizers, pMDIs, and dry-powder inhalers (DPIs) still need advancement for the perfect delivery of macromolecules. It is vital to seek approaches that can address issues such as deposition, the fine particle fraction, stability, shelf life, patient-friendliness, and multiuse application.¹¹³ In the current scenario, it is of paramount importance to address these issues concerning pulmonary delivery systems to enable both site- and cell-specific delivery.

Discussion

The present review suggests the possibility of COVID-19 therapy or prophylaxis via the pulmonary aerosolized or nasal delivery of serum antibodies collected from COVID-19-recovered patients. We also present an overview of the clinical outcomes of convalescent plasma containing antibodies and the pharmaceutical aspects of the antibody-based formulations that are indicated for respiratory diseases. Furthermore, we review the proposed methods to overcome the difficulties allied to mAb administration as an aerosol formulation and sum up the different preclinical and clinical investigations on inhaled mAbs.

The current era of COVID-19 has necessitated an evaluation of the efficacy of convalescent plasma therapy. The salient investigations into this topic are five clinical trials on the use of human anti-SARS-CoV-2 plasma for the prevention and treatment of COVID-19. One of these studies was conducted by Ahn and others, who reported the successful use of convalescent plasma therapy for two patients admitted to the hospital on day ten and day six in Korea with severe COVID-19 and ARDS, respectively (day 22 and day seven of symptom onset). The clinical, biochemical, and radiological tests in that research showed improvements and were negative for SARS-CoV-2 RNA.¹¹⁴ Another investigation was performed by Figlerowicz and colleagues, who reported the success of convalescent plasma therapy in resolving severe COVID-19 symptoms in a 6-year-old patient. Their pediatric patient had developed aplastic anemia, which proved refractory to treatment in the first five weeks of hospitalization.¹¹⁵

Convalescent plasma therapy can be successfully applied through the collection of blood samples and transfusion methods from well-recognized centers globally. The use of plasma collected from recovered patients for the treatment of severe COVID-19 has been approved by the US Food and Drug Administration (FDA) in the United States. The guideline stipulates that the transfused plasma be procured from negatively tested donors for COVID-19 prior to day 28 of clinical recovery and day 14 of recovery without any symptoms. A rise in the number of candidates for plasma donation has been reported in tandem with an increase in the recovery rate from COVID-19 infection.¹¹⁶ Be that as it may, various obstacles complicate the procurement of convalescent plasma. Several investigators have recommended apheresis instead of whole-blood donation intending to augment convalescent plasma harvest inasmuch as this technique promotes the collection and processing of the critical fraction of the blood required to produce convalescent plasma. It is to be noted that a solo donation renders approximately 400 to 800 mL of plasma, which yields two to four units of convalescent plasma for transfusion. The samples are preserved in keeping with the standard methods, which require adherence to the regulatory guidelines for the testing of most common transfusion-transmitted infections such as human immunodeficiency syndrome and hepatitis B and C viruses. In pregnant women volunteering to be donors, it is vital for the testing of human leukocyte antigen antibodies to rule out the possibility of transfusion-related acute lung injury.

Regarding the possible adverse effects, while Joyner and colleagues reported none in a large national, multicenter cohort that received convalescent plasma therapy,^{117, 118} risks such as allergic reactions were associated with plasma transfusion. Plasma contains procoagulants, whose aftereffects are unidentified in COVID-19, due to which treatment for COVID-19 requires utmost care in patients with acute thrombotic events. Considering such probable risks, studies are warranted to verify these observations concerning convalescent plasma transfusion for the therapy of COVID-19.

According to the FDA's guidance, antibody testing has its own challenges. Meticulous vetting is mandatory for the qualification of donors or the manufacture of therapeutic agents through tests. The matters are even more complicated by the uncertainty as to which antibodies are optimally effective in the treatment of COVID-19. Neutralizing antibodies are likely to interact

better with the sites of COVID-19 responsible for binding with the ACE2 receptor. In the clinical laboratory setting, neutralizing antibodies have been found unresponsive to high productivity screening, which diminishes their availability. In contrast, limited data are currently available on the enzyme-linked immunosorbent assay (ELISA) quantitative assay and have not been rigorously validated commercially. It might be possible to capture allo- or autoantibodies present in patients suffering from COVID-19 with delayed severe complications by designing an encapsulated ELISA featuring a plate coated with recombinant ACE2 or its complexes with the S protein or its S1 subunit as a specific receptor, thereby permitting the measurement of the kinetics of these antibodies during the pathological evolution. Such antibodies are expected to be alloantibodies, if they are present and stimulated by the amalgamation of the viral protein. To capture antibodies or antigens as per requirement, researchers could design a similar model on an affinity column matrix. However, in practice, what we need urgently is not easily matched with what we want.¹¹⁹

As COVID-19 cases increased drastically worldwide, reports pointed out that SARS-CoV-2-specific IgG antibodies passively transferred by transfusion could be utilized as a therapy. This method of treatment might help neutralize the virus and stimulate the complement system. Still, the association between total SARS-CoV-2 antibodies and neutralizing anti-SARS-CoV-2 antibodies has yet to be clarified. There are still some lacunae about the testing and use of the majority of favorable total antibodies or subclasses of antibodies (i.e., IgM, IgG, and IgA) and the specific antigen for the S protein.^{120, 121} Nonetheless, Amanat and colleagues developed an assay using recombinant antigens derived from the SARS-CoV-2 S protein, which could evaluate both strong reactivity against these immunoglobulins (e.g., IgG3, IgM, and IgA) and low cross-reactivity for testing other human coronaviruses. This method conferred the screening and detecting of antibodies at an early stage of COVID-19 (i.e., even two days) following the onset of symptoms in human plasma/serum. It is deserving of note that the investigators posited that this test could be executed in the absence of pathogens and that it could promote the detection of different classes of antibodies.¹²⁰ The method demonstrated in that study is the most sensitive and distinct for the estimation of SARS-CoV-2 antibody titers, whereas a few studies have demonstrated the detection of SARS-CoV-2 antibodies between eight and 21 days after the onset of symptoms.^{121, 122}

Observations from China showed a high titer of anti-SARS-CoV-2 antibody in convalescent plasma collected at least 14 days post symptom recovery. Similarly, Li and others developed a rapid immunoassay capable of concurrently identifying within 15 minutes both IgM and IgG antibodies against SARS-CoV-2 infection in human samples. The test afforded the possibility to diagnose the stage of infection for prompt medical care. Their results suggested sensitivity of 88.7% and specificity of 90.6% in the detection of IgM and IgG together. Their study also reflected the utility and precision of the IgG-IgM combined assay when compared with IgG or IgM alone for the fast detection of carriers, symptomatic or asymptomatic for SARS-CoV-2 infection.¹²³ Hence, mAbs, hyperimmune globulin products obtained from fractionated plasma, could be used as an alternative substantial treatment that is instantly available to treat and prevent COVID-19 infection.

Although injections are the most common means for the administration of these protein and peptide drugs, other alternative routes including oral, buccal, intranasal, pulmonary, transdermal, ocular, and rectal have also been tested by researchers with erratic rates of success.¹²⁴⁻¹²⁹ With their rapid elimination from the circulation because of enzymatic degradation, renal filtration, uptake by the reticuloendothelial system, and accumulation in non-targeted organs and tissues, the use of several polypeptides is restricted.¹³⁰ To improve pharmacokinetic properties and enrich the exposure of protein therapeutics, researchers have developed different technologies for patient comfort such as less frequent administration, greater convenience, and improved efficacy. Protein fusion technologies, via molecular engineering, seek to enable a protein to combine with the long serum persistence of an endogenous protein and the biological activity of the protein of therapeutic interest. Fc-fusion technology utilizes the recycling system of the FcRn receptors to generate molecular entities (the Fc domain of antibodies) with an increased half-life.¹³¹ The construction of human serum albumin or transferrin fusion proteins is an alternative approach to protein fusion technologies to augment the time action of protein therapeutics.^{132, 133} A large polyethylene glycol (PEG) moiety can be covalently or reversibly attached to the protein through a reactive group like amines, thiols, or carboxylic acids by using PEGylation technology. This technology is advantageous in increasing molecular stability, changing the volume of distribution, reducing immune reactions, and decreasing the polymers

attached to the protein globule, thereby creating steric hindrance for the interaction between protected polypeptides and the active sites of proteases, opsonins, and antigen-processing cells.^{134, 135}

The disease management of patients with pulmonary diseases needs aerosol therapy, whereas the treatment of patients with COVID-19 requires a different approach. As it is imperative during the pandemic to assume that all patients may be infected, good personal protection and aerosol administration practices should be applied. Further clinical studies are needed given the lack of proper information and guidance on how to administer aerosolized medications to those infected with COVID-19. Modifications in the dose, frequency, and delivery techniques may be required in the effective delivery of aerosolized medications to these patients during aerosol therapy.

The spread of the novel coronavirus can aggravate in patients with COVID-19, when aerosolized medications are prescribed. Despite the fact that vaporized treatment may be a backbone strategy utilized to treat pneumonic diseases in domestic and healthcare settings, it incorporates a potential for criminal emanations amid treatment due to the era of mist concentrates and droplets as a source of respiratory pathogens. Fugitive emission is defined as aerosols, which have been released from the aerosol device during the patient expiration. These aerosols are not breathed in by the patient but pass into the environment. This has been a genuine concern for caregivers and healthcare experts, who are vulnerable to an unintended inward breath of these aerosols while giving treatment. This paper discussed the methods for the delivery of aerosolized medications to mild, less-intensive, and intensive patients suffering from COVID-19 and simultaneously to introduce a well-defined procedure for the protection of persons who deal with patients and are exposed to exhaled droplets during aerosol therapy.¹³⁶

There are some limitations to this proposal. The implementation of convalescent plasma therapy requires clarification for the optimal concentration of neutralizing antibodies and treatment programs. The pathological changes leading to the aggravation of cytokine responses at the stage, where the treatment modality is needed is still a matter of concern (early vs. intermediate-late stages of the cytokine storm reaction phase associated with ARDS or other severe disease complications). Likewise, emphasis on employing plasmapheresis in immunocompromised subjects, especially those with hematological malignancies, has yet to be

explained. Given the increased mortality and morbidity rate observed in the current scenario of COVID-19 infection, it is vital to recognize the most vulnerable population with the objective of suggesting new therapeutic entities.

The risk of infection due to the administration of aerosolized formulations is lower than that due to asymptomatic COVID-19–infected individuals visiting treatment centers without facemasks. It follows that inhaled medications have the potential to be administered at all stages of COVID-19 infection. Proper personal protective equipment must be donned by healthcare personal during the administration of aerosolized formulations to patients, so as to minimize the risk during patient procedures.¹³⁷

Furthermore, the pulmonary or nasal route of administration can be explored with a view to enhancing the bioavailability of sera containing Abs and limiting their harmful effects through the systemic circulation.

Conclusion

COVID-19 is currently a hazard to health worldwide, as still no specific antiviral therapy is available. In the present review, we propose the delivery of serum antibodies collected from COVID-19–recovered patients using a pulmonary aerosolized formulation or a nasal drop in the treatment and prophylaxis of COVID-19. According to previous reports, convalescent plasma is well tolerated and contains neutralizing antibodies responsible for the disappearance of clinical symptoms and viremia. Based on the information reported in the present review, it can be concluded that convalescent plasma containing antibodies can serve as a promising therapy for COVID-19 treatment and can help perform randomized clinical trials.

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