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The Use of Nebulizer Medications as a Possible Treatment for COVID-19

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The Use of Nebulizer Medications as a Possible Treatment for COVID-19

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The Use of Nebulizer Medications as a Possible Treatment for COVID-19

Submitted in partial fulfillment
of the requirements
for the Murray State University Honors Diploma

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To my beautiful godchildren Alora and Memphis,
may this work bring hope for a safer and brighter
future for you both

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Preface

For the past few years, the COVID-19 pandemic has been the focal point in healthcare and research. This disease has permanently changed daily life and left a historic impact on the world. Most people have felt the effects of this pandemic either directly, via infection, or indirectly, via change in workflow, financial impact, etc. The main question for this virus still remains today; how do we treat this illness effectively? While many ideas are being tested and suggested, a definite answer has yet to be procured. Vaccine rates are climbing on a daily basis, serving as the first and most beneficial form of prevention when it comes to spread and severity of infection. However, as new variants continue to mutate into existence, we are left wondering how long this virus will be an active threat. For this purpose, nebulizer medications may serve as an effective and long-term option to treat positive patients both in healthcare settings and at home. The main medications of ipratropium bromide and albuterol sulfate and their premixed combination have shown benefit in patients with other respiratory conditions such as COPD and asthma. Given the correlation of symptoms between many of these diseases and COVID-19, there stands plausible support for the use of these medications for treatment of a viral SARS-CoV-2 infection. If these medications could sucessfully treat COVID symptoms, then perhaps these medications hold stake as a possible option. This would help to ease the strain on the healthcare system and bring peace of mind to those with growing concerns for their health and safety.

Chapter 1: Lung Receptor Anatomy and Biology

In order to dive further into the benefits of this method of treatment for COVID-19 patients, it is imperative to have knowledge of the basic systems that are involved. Both SARS-CoV-2 and nebulizer medications utilize separate systems, and it is important to understand each systems' function to grasp the correlation. We will see that not only are the systems targeted in individual manners, but that the systems themselves are interconnected in their biological pathways. First, we look at three receptor systems and their functions in the lungs.

1.1 Beta-Adrenoceptors in Lung Tissue

One of the more common receptor systems found throughout the lung's tissue is the β-adrenoceptors. These receptors are highly concentrated throughout the entirety of the smooth muscle tissue in the upper and lower airways. An article from the National Heart and Lung Institute from Imperial College in London states, "The density of β_2 -receptors in airway smooth muscle does not change at different airway levels, so that bronchioles have a similar density to large airways" (3). This shows that regardless of location in the lung system, there are β-adrenoceptors present. In addition to their high concentration, they are also broken down into a few main subtypes. The two subtypes of beta receptors are β_1 and β_2, and studies show that 70% of the β-adrenoceptors in the lungs are β_2 (3). This subtype is located in smooth muscle, epithelium, and submucosal glands while β_1 receptors are located primarily in the submucosal glands. The two subtypes are also located in the alveolar wall with the aforementioned article by Peter J. Barnes noting, "There is a uniform distribution of β-receptors on the alveolar wall with a ratio of $\beta 1$: $\beta 2$ receptors of 2:1" (3). While β_2-receptors are primarily responsible for the regulation of the bronchial constriction response, the β_1 receptors work on regulating mucus

production in the alveoli (10). The mechanism by which the β_2-receptor causes smooth muscle relaxation is well outlined by a *Pharmacological Reviews* article. Matera et al (10) mentions that activation of β_2- adrenoceptors on airway smooth muscle (ASM) generates intracellular cAMP by adenylate cyclase activation. This in turn activates its effector molecules, cAMP-dependent protein kinase A (PKA), which is a Rap1 guanine nucleotide exchange factor (10). The cAMP wave also downregulates Rho and causes calcium to be sequestered in the smooth endoplasmic reticulum. This in combination with the phosphorylation of regulatory proteins leads to ASM relaxation in the tissue (10). This effect translates into other forms as the more common β_2-receptor is located in more areas than just lung tissues.

1.2 Alternate Functions for Beta2-Adrenoceptors

In addition to lung tissue, other systems of the body possess these same receptor types. Most prevalent being the white blood cells of immune system. While our bodies are well equipped to fight viruses and other pathogens that may enter our system, the side effects of the immune response can be debilitating for a patient. The key result of white blood cells fighting off an infection is the inflammatory response caused in the body. The β_2-adrenoceptors in the ASM are also found on these immune system cells. The inflammatory response generated from by white blood cells can be downregulated via stimulation of the β_2-receptors. Peter Barnes claims, "Inflammatory cells that are involved in asthma and COPD, including eosinophils, neutrophils, T lymphocytes, and macrophages, all express a low number of β_2-receptors. In vitro, β_2-agonists have been shown to inhibit the release of inflammatory mediators from these cells" (3). Here we can see that these receptors have a similar function in white blood cells as with ASM. This process of de-inflammation leads to a cascade of other benefits, one being a reduction in plasma exudation. Plasma exudation refers to the leaking of plasma out of postcapillary venules into the

surrounding space. This is usually the result of acute inflammation in tissues. Just as in white blood cells, β_2-receptors are also located on the endothelial cells in these post-capillary venules. Stimulation of these receptors helps to prevent the leaking of fluid and edema in nearby systems (3). The reactions that stimulate the beta-adrenoceptor system are crucial when taking into account treatment options for lung diseases. In addition to this receptor system, another exists that has important cross correlation to the β-adrenoceptors.

1.3 Muscarinic Acetylcholine Receptors (mAChRs)

Another key receptor system in the lungs is the muscarinic acetylcholine receptor system. This receptor type is typically associated with its function in the parasympathetic nervous system. Airway smooth muscles receive parasympathetic input from the vagus nerve, and the activation of the vagus causes smooth muscle contraction. The substrate for these receptor types is acetylcholine (ACh), and its binding to its target receptor can lead to this negative symptom and impaired lung function. The aforementioned work by Matera et al. states, "Acetylcholine (ACh) is the neurotransmitter in the parasympathetic nervous system, both in the ganglia and at the neuroeffector junction. ACh activates muscarinic acetylcholine receptors (mAChRs) postsynaptically on ASM and mucous glands to elicit bronchoconstriction and mucous secretion, respectively" (10). As with the beta-adrenoceptors, the mAChRs are broken down into a few subtypes. The main subtypes are M_1, M_2, and M_3 mAChRs (3). The receptor most related to the ASM constriction is the M_3 mAChRs. When ACh binds to an M_3 receptor in the lungs, phospholipase C will generate the second messenger IP_3. This leads to a calcium release from the sarcoplasmic reticulum and the contraction of ASM via calcium-calmodulin dependent myosin light chain kinase (10). The other receptor types use the same substrate but serve slightly

different functions. The M_2 receptor subtypes are also referred to as inhibitory muscarinic receptors or auto receptors. These subtypes are mainly located in the presynaptic terminals of the post ganglionic parasympathetic neuron while some can be located directly on the ASM. On the post-ganglionic nerve, they inhibit the release of ACh from the nerve to the ASM (3,10). When these receptors bind ACh they reduce the release ACh on to other muscarinic receptors in the ASM. However, receptors on the ASM serve a slightly different function. A manuscript from the Oregon Health and Science University says that these receptors, "…inhibit relaxation induced both by β-adrenoreceptor agonists and adenylyl cyclase activation with forskolin. Thus, M_2 receptors contribute to smooth muscle contraction by functionally antagonizing Gαs-induced relaxation" (4). In the ASM the M_2 receptors promote the constriction of the airway smooth by blocking other methods of dilation. The final receptor subtype is the M_1 subtype. These, like the M_2, are located in two different locations. First, they are in the postsynaptic terminals of the post ganglionic parasympathetic nerve. In this position they serve to help stimulate neurotransmission. If this receptor were blocked so that ACh could not bind, there would be a possible reduction in neurotransmission of the vagus nerve signal. This would reduce the amount of ACh released onto the ASM and reduce the constriction response in ASM (4,10). Second, M_1 subtypes are also found in the airway submucosal glands along with M_3 subtypes. In these glands, ACh binding causes both subtypes to secrete mucins and fluid. Buels and Fryer note this designated function by stating, "In submucosal glands, muscarinic receptors are found on both serous cells that secrete fluid and mucous cells that secrete mucins. Both M1 and M3 receptors are present in human and animal submucosal glands" (4). Given the variation in subtypes and their functions, it is also important to note the methods by which the substrate, ACh, is presented to the receptor.

1.4 Stimulation Methods in Muscarinic Receptors

With the multiple subtypes of receptors, their specific location in the lung determines how they will be stimulated. There are two manners in which the mAChRs are stimulated with ACh. The first is stimulation through cholinergic nerves and the parasympathetic nervous system. However, the level of innervation of the nerve system changes throughout the lung tissues. The article by Barnes notes, "Postganglionic fibers then innervate airway smooth muscle and submucosal glands. Vagal innervation of the airways is predominantly in large airway and diminishes peripherally with no motor innervation of small airway and lung parenchyma" (3). In addition, the concentration mAChRs begins to decrease into the smaller airways of the lungs. Given this decrease in the nervous systems ability to stimulate, another method of ACh delivery occurs. The ACh needed to activate the mAChRs can also be produced from other cells in the tissues of the lungs. This is done by choline acetyltransferase (ChAT). Both epithelial and inflammatory cells possess transferase enzymes, and enzyme production increases as the cell is exposed to inflammatory factors such as tumor necrosis factor alpha (TNF-α) (3). These two different stimulation methods are shown in **figure 1 (3)**:

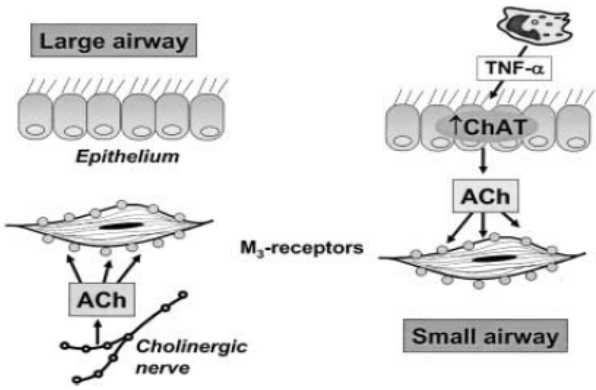


Figure 1: Methods of mAChR Stimulation. Adapted from "Distribution of receptor targets in the lung," by P.J. Barnes, Proceedings of the American Thoracic Society, 1(4), 345-351. *(3)*

These two pathways are how bronchoconstriction occurs via this receptor type. They work in unison to alert the body to stress and infection no matter which part of the lungs are affected. While both these receptor systems are different, they are also strongly interconnected.

1.5 Muscarinic and Beta-Adrenoceptor Cross Correlations

The correlation between both receptor systems serves as a building block in the treatment of lung diseases. Multiple connection points in their individual pathways lead to cross-stimulation and inhibition. Both of these receptor pathways work to stimulate second messengers in the cell. The muscarinic system works to increase intracellular calcium and activate protein kinase C (PKC). These events promote contraction of ASM via the calcium-calmodulin dependent myosin light chain. On the other hand, the beta-adrenoceptor system works to promote the formation of the second messenger cAMP and promote the relaxation of ASM. The activation of PKC serves the function of desensitizing the β_2-receptor. Given that the β_2-receptor is a G-protein coupled receptor, PKC will phosphorylate its G_s subunit leading to a desensitization of the receptor. This reduces the beta adrenoceptor's ability to generate the second messenger for relaxation via its downstream pathway (10). On the contrary, the beta-adrenoceptor pathway is shown to lower the concentration of intracellular calcium via its sequestration caused by cAMP. The cAMP-dependent protein kinase A (PKA) activated by the adrenoceptor pathway is also thought to reduce the production of IP_3 , the second messenger in the ASM contraction of the muscarinic receptor system. In addition, the stimulation of the beta adrenoceptors leads to the prevention of release of ACh into the synaptic space by activating calcium-activated potassium channels in the postganglionic neuron. Matera et al. states, "It is likely that these channels reduce

the concentration of Ca^{2+} by hyperpolarizing the cell membrane and, consequently, inhibit the release of ACh" (10). These different inhibition relationships show how one system effects another to alter the response of lung tissues. A brief synopsis of these relationships is given in **figure 2 (10):**

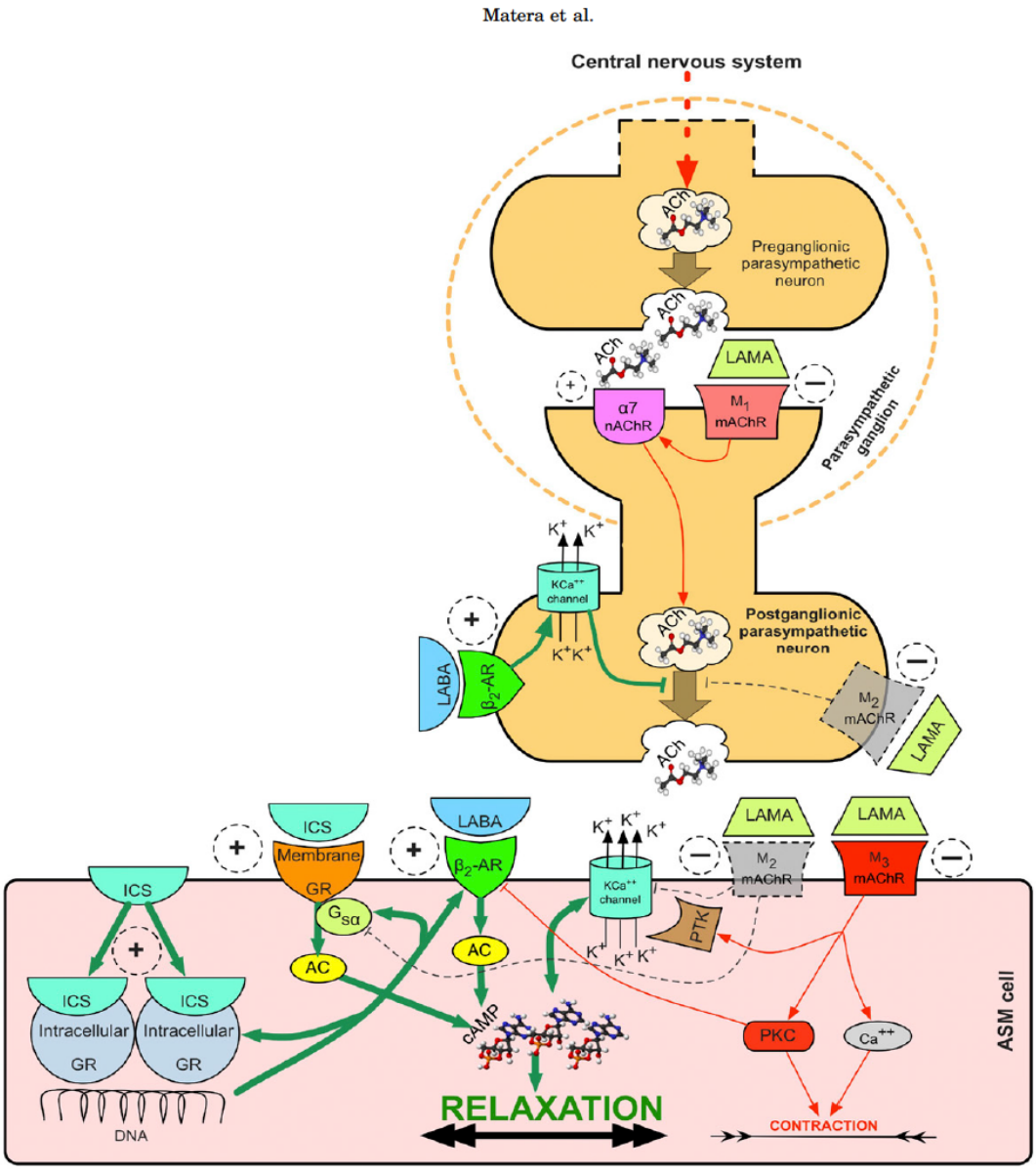


Figure 2: Biological Pathways: Response of Receptor Inhibition and Stimulation
Note: LABA (LONG ACTING BETA AGONIST) and LAMA (LONG ACTING MUSCARINIC ANTOGONIST.

Adapted from "Pharmacology and Therapeutics of Bronchodilators Revisited," by M. G. Matera, C. P. Page, L. Calzetta, P. Rogliani and M. Cazzola. Eric L. Barker, Pharmacological Reviews January 2020, 72 (1) 218-252. (10)

The final receptor system to take into consideration is the one that SARS-CoV-2 works on directly.

1.6 The Angiotensin Receptor System

The renin-angiotensin system (RAS) is commonly categorized as a system for inflammation and de-inflammation. The system utilizes the combined system of converting enzymes and angiotensin. These enzymes are expressed predominately in the cardiovascular and pulmonary systems. Renin is released from the kidneys in response to a low sodium intake and sympathetic stimulation. This leads to the formation of angiotensin peptides that serve various functions. The main focus is placed on angiotensin II (AngII). When renin leads to the formation of different angiotensin peptides, angiotensin converting enzyme (ACE) converts the local angiotensin one (AngI) into its more detrimental form, AngII. An increase in AngII is associated with inflammation in tissues and an elevated vascular constriction (12). Another converting enzyme receptor, angiotensin converting enzyme two (ACEII), breaks down AngII and cleaves this angiotensin into angiotensin 1-7. With AngI, ACEII breaks this down into angiotensin 1-9 (9). This breakdown of the angiotensin proteins promotes vasodilation and de-inflammation. This system is important in understanding how the SARS-CoV-2 infects a host.

Chapter 2: SARS-CoV-2 In the Lungs

Next, we explore the process by which SARS-CoV-2 enters the body and begins the process of infection. SARS-CoV-2's detrimental effects can send patients to the hospital and even cause fatality. So, what are the key issues with a COVID diagnosis and what symptoms do we need to treat to protect patient health? Transmission is important for understanding how this process starts.

2.1 SARS-CoV-2 Transmission and Deposition

Most individuals understand that SARS-CoV-2 is transmitted via aerosol and the air. The term aerosol is defined as solid particles or liquid droplets with small diameters of about a few nanometers and micrometers (19). SARS-CoV-2 has been found to spread quickly from person to person via this method. This is because aerosols released from individuals begin to spread out as they travel outwards. This results in the intake of the virus through inhalation. Some of the main methods of transmission from an infected host are coughing, sneezing, breathing and speech. These actions produce aerosol particle of various sizes. The same article by Zuo et al. makes an estimate to the number of virions in an aerosol. It states, "COVID-19 patient sputum contains 10^6–10^{11} viral RNAs per milliliter, although this number "can overestimate infectious virions". If sputum matter is aerosolized as 5 μm particles, then the average number of virions per aerosol, using 10^{11} virions/mL, is no greater than 50" (19). They go on to state that the vast majority of the aerosols, approximately 67%, released from an infected patient contain at least one active virion if not more. The various sizes and properties of these individual particles play a role in the virus's deposition into the lungs. A smaller particle size will allow for better

sedimentation into the lungs. The particle size effects what area of the lungs the virus will be located in. **Figure 3 (19)** shows how the different aerosol sizes determine that location:

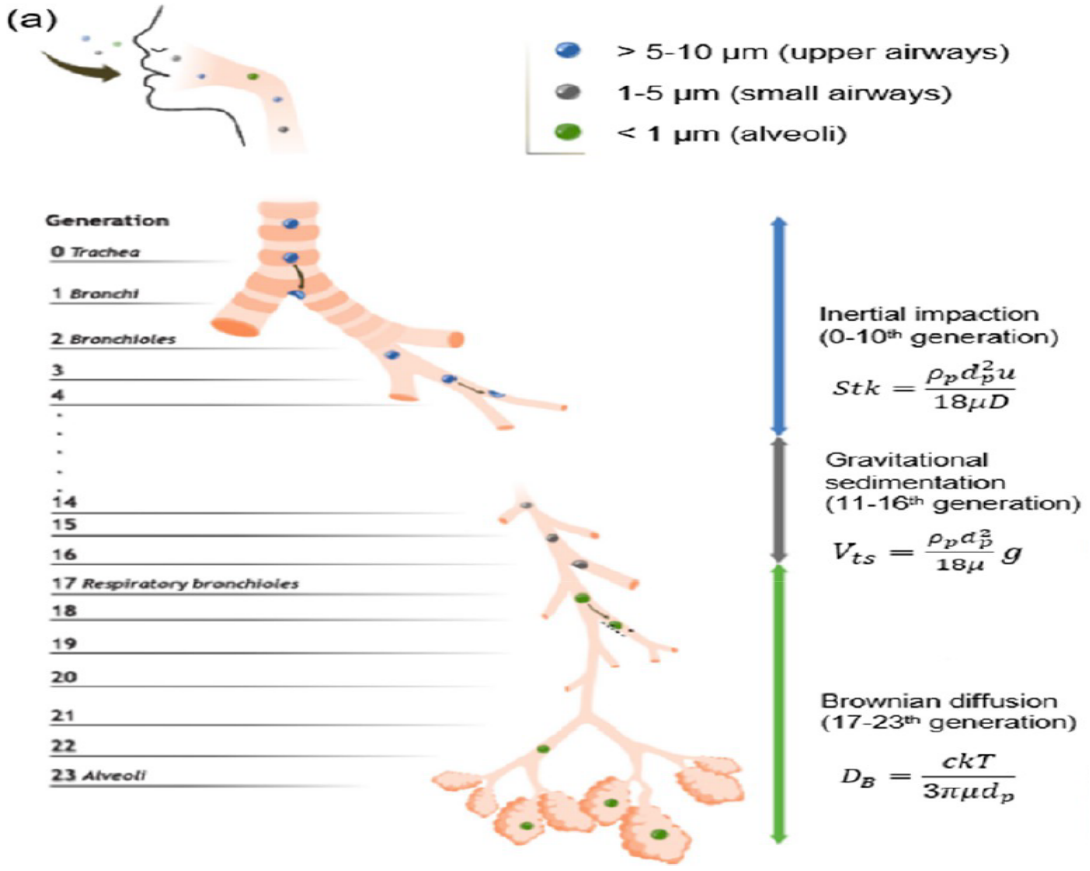


Figure 3: Airway Deposition by Particle Size. Adapted from "Airborne transmission of COVID-19: aerosol dispersion, lung deposition, and virus-receptor interactions," by Zuo, Y. Y., Uspal, W. E., & Wei, T. (2020), ACS nano, 14(12), 16502-16524. (19)

As shown, SARS-CoV-2 location in the lungs is based on the size of the particle the virion travels in and can be lodged in the alveoli, large airways or small airways. Regardless of the location of the infection, the process by which SARS-CoV-2 enters the lungs tissue is the same.

2.2 SARS-CoV-2 Infection Process

The RAS system serves as the entry point of SARS-CoV-2 into the body. The SARS-CoV-2 virus uses its spike protein to latch onto a target receptor in the lung tissue after inhalation. The spike protein has a strong binding affinity to the ACEII receptor of the RAS system. It uses this receptor to gain entry into the lungs cells and begin replication (12, 19). While the virus may be localized to any point of the lung system, and ACE receptor will more than likely be present near that location in an epithelial cell. As SARS-CoV-2 enters the cell, it begins to pass through its replication cycle and use the host cells machinery to create more active virions. After lysing the cell, the virions spread to other areas of the lungs and other cells. As this process continues, more sections of lung tissue become infected with the virus and the replication process increases exponentially. The virus moves from the airways deeper into the tissues of the lungs. This creates a rapid onset of infection once the process beings. SARS-CoV-2's infection process is a double edge sword. When the virus uses an ACEII for entry, it consequently lowers the expression of ACEII via the RAS pathway activation. This increase is AngII results in system inflammation and constriction (12). The spread of SARS-CoV-2 also leads to alveolar infection which is where the most severe symptoms begin.

2.3 Symptoms of Infection

As the infection of SARS-CoV-2 moves through the lung system, at some point it reaches the alveolus. The type II pulmonary alveolar epithelial cells are some of the main cells that express ACEII the most, leaving them a prime target for SARS-CoV-2 entry (16). As this area

becomes infected, problems develop with breathing function and oxygen exchange. A diagram of these issues is outlined in **Figure 4 (19)**.

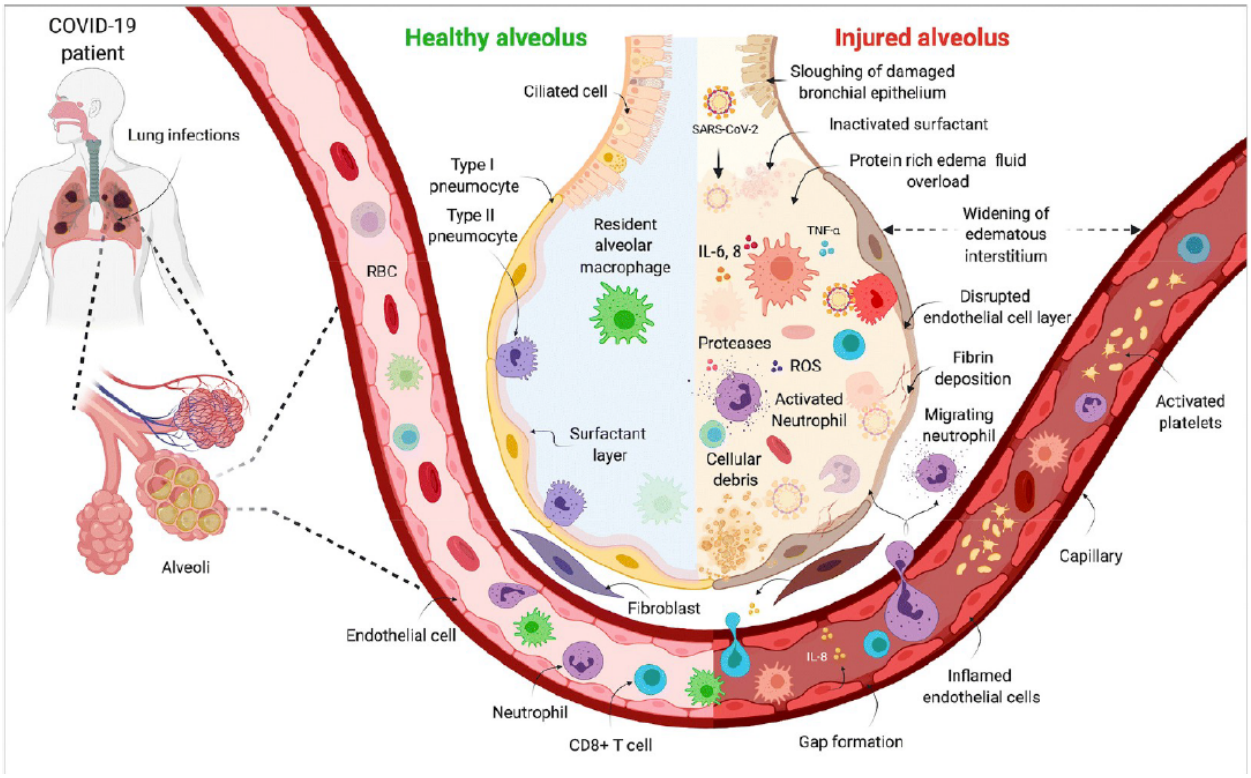


Figure 4: A Comparison of Healthy Versus Infected Alveoli. Adapted from "Airborne transmission of COVID-19: aerosol dispersion, lung deposition, and virus-receptor interactions," by Zuo, Y. Y., Uspal, W. E., & Wei, T. (2020), ACS nano, 14(12), 16502-16524. (19)

The issues produced by COVID-19 are compounded in nature. Not all patients will find a way into the hospital from a SARS-CoV-2 infection, but the magnitude of damage and symptoms is the final determinate. The first systematic response is the activation of multiple white blood cell types. From mast cells to neutrophils, the innate immune system activates to defend the body from the invading virus. This storm leads to intense inflammation in the lungs. An article in *BioEssays* by Natesan Vasanthakumar gives light to this by stating, "Cytokine storm and hyperinflammation occurs in COVID-19 irrespective of lymphopenia. Considering the cytokine

storm and potential activation of the NLRP3 inflammasome, it is likely that immune hyperactivation occurs in COVID-19" (16). One of these cytokines is tumor necrosis factor alpha (TNF-a). As mentioned before this inflammation factor is used as a signaling molecule in the muscarinic acetylcholine system. This leads to an increase mucus production and bronchoconstriction in SARS-CoV-2 infected patients. "Increased mucus aggregation has been noticed in the distal airways and alveoli in the postmortem study of COVID-19 patients", notes Vasanthakumar (16). The mucus production is also increased by the presence of white blood cells which work to produce ACh via their ChAT. This viral infection of the alveolus also produces a breakdown of the epithelial cell structure. This allows for the fluid secretion in the interspace between the post capillary venules and edema in the alveolus. It also forms cellular debris build up that prevents proper airway exchange. Oxygen exchange and saturation rates drop as more alveoli become infected and damaged. The combination of sluffing cells, mucus, fluid, and constriction can be devastating for an infected patient. This complexing issue can lead some more severely infected COVID patients into acute respiratory distress syndrome (ARDS). Approximately 5% of COVID-19 patients progress to ARDS (16). This is made worse by the downgraded expression of the ACEII by SARS-CoV-2 in its infection process. ACEII has been shown to help prevent the progression into ARDS by its anti-inflammatory effects (9). This risk of ARDS results in a high level of concern in regards to mortality. Many patients who develop ARDS end up being placed on a ventilator to keep them breathing. A treatment option must be one that supports a decrease in mucus production, fluid secretion, cytokine storm and bronchoconstriction. The answer to these needs lies in the use of nebulizer medications.

Chapter 3: Nebulizer Medications and Their Benefits

Plausible treatment for SARS-CoV-2 infections can be found in nebulized treatments. The direct action of the medicinal aerosol to infected lung tissues is important for opening airways and promoting better breathing. Nebulizers create this aerosol via nebulization which is inhaled by the patient. Two medications in particular can be prescribed at low cost and with ease of access to patients to stabilize their breathing during an infection. Looking into the benefit and functionality of the nebulizer and its medications proves its benefit as a treatment option.

3.1 Nebulizer Types and Their Function

The nebulizer is a device that takes liquid solutions and converts them into an aerosol. One type of nebulizer is the jet nebulizer. This device consists of the nebulizer console, tubing to pass the airflow, and mouthpiece where the medicine solution is placed. When the machine is activated, compressed air, or another gas, is passed through the tubing from the nebulizer. This gas passes by the solution, draws it into the stream, and breaks it down into aerosol particles (2). Baffles are used to take any large droplets and return them back to the mouthpiece cup for re-nebulization. This is one of the more common nebulizers used to deliver nebulizer medications to patients. Another type of nebulizer is the vibrating-mesh nebulizer. This nebulizer is similar but uses a different tactic for generating the aerosol. A small mesh plate with small holes is vibrated with electricity. The liquid is passed from a solution reservoir raised above the plate to be broken into aerosol via the vibrating plate (2). The medication is then drawn into the attached mouthpiece to be passed to the patient on the inbreath. Even with the different styles of nebulization, both of these serve to generate an easy to inhale form of the medication. This aerosol is delivered directly into the patient's lung tissue and can reach into the secondary small

airways. There are multiple different medications that can be delivered via nebulization, but it is important to note those that affect receptor systems involved with a SARS-CoV-2 infection.

3.2 Ipratropium Bromide and Albuterol Sulfate

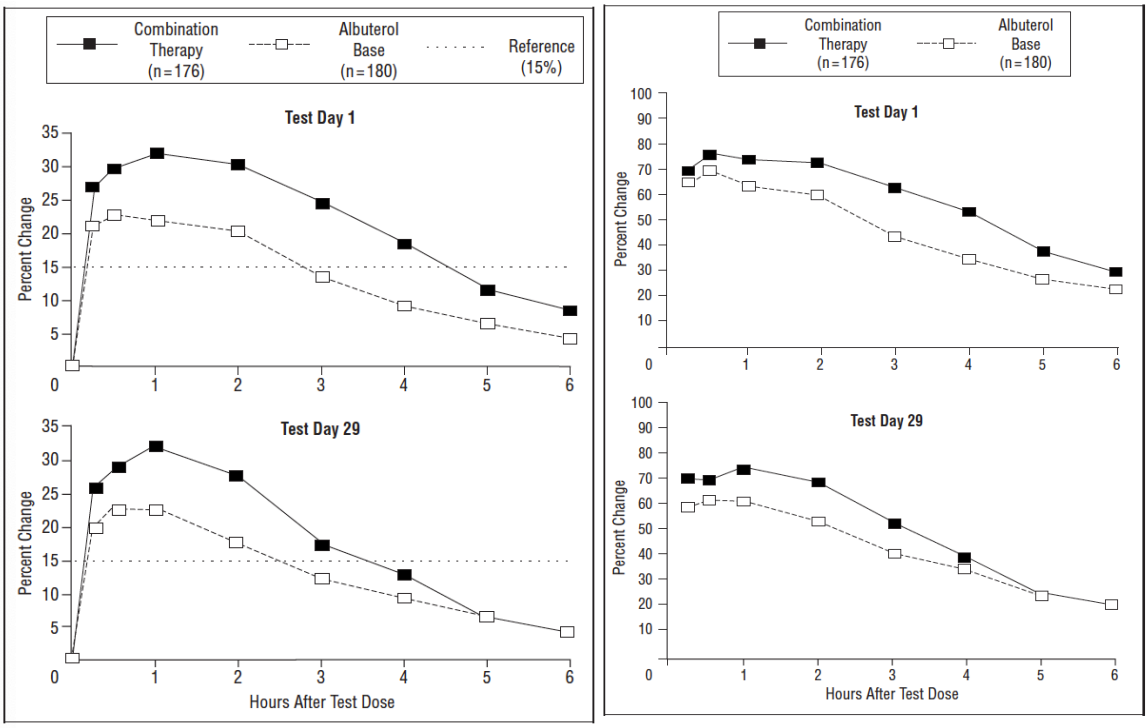
Ipratropium Bromide and Albuterol Sulfate are two nebulizer medications that effect the aforementioned muscarinic receptor and beta-adrenoceptor systems, respectively. These two medications that act on each system in opposite ways. For ipratropium bromide, it serves as a muscarinic receptor antagonist. This means the binding of this medication to any muscarinic receptor subtype means that it will turn off and de-activate these receptors (15). This means that the binding of acetylcholine to these receptors will be blocked. This medication will lead to a decrease in the expression of mucus as well as a decrease in bronchoconstriction. As discussed previously with the multiple M-type receptors, halting these receptors functions will promote the relaxation of ASM that has been constricted by both vagus nerve integration and white blood cell acetylcholine. This medication will also promote the blocking of the M_2 receptors which stops inhibition of the ACh from the postganglionic neuron, causing it to release ACh. While the ACh is building up in the synapse of the ASM, the M_2 and M_3 receptors on the ASM are also blocked and cannot accept the ACh, and therefore bronchoconstriction is prevented (15). For the Albuterol, it has an opposite effect on its target system. This medication serves as an agonist to the beta-adrenoceptor system (5). This means that the medication will activate or stimulate these receptors and cause them to perform their function. This medication will promote ASM relaxation by the adrenoceptor pathway. Stimulation of these receptors will not only promote airway opening but also downregulate the inflammatory response of white blood cells and prevent the secretion of fluid into the airways. Both these medications have been shown to

promote the opening of the airways and prevent patient's from not being able to breath. A study of both meds from the Respiratory Medicine Unit at City Hospital in Nottingham, UK, outlines how these medications effect patient breathing. The studies are done in terms of force expository volume (FEV), which means the amount of air a person can exhale on a forced breath, and PD_{20}, which is a 20% drop in FEV after exposure to a provocative dose of histamines meant to cause asthmatic reactions in patients. Higgins et al. states, "In the asthmatic subjects mean PD_{20} changed from 0.80 μmol to 4.75 μmol following salbutamol (Albuterol Sulfate) and from 0.67 μmol to 1.06 μmol following ipratropium bromide. The mean increase in PD_{20} was significantly higher with salbutamol than with ipratropium (2.26 vs 0.84 doubling doses, $p<0.05$)" (7). This means it took a higher dosage of the histamines to cause the patients to lose air capacity in the lung tissue with both medications. Their FEV would not drop by 20% without a more concentrated dose of the stimulant. The study showed similar results in patients with chronic bronchitis with both medications still stabilizing the air compacity of the patients for target FEV values (7). Given their strong effect in treating airway diseases, and the systems the virus effects, COVID-19 would be treatable with these medications. It is also important to note that combination therapy of Duoneb is even more effective than the medications individually.

3.3 Combination Therapy Versus Individual Medications

Ipratropium Bromide and Albuterol Sulfate are medications that come in a mixed form besides their individual treatments. This medication combo is even more effective at holding a patient's airway capacity and increasing the FEV of COPD patients. This is because the mixed combination acts on both muscarinic receptor and beta-adrenoceptor systems at the same time via antagonism and agonism, respectively. Dr. Sammy Campbell from the Department of

Medicine and Respiratory Sciences Center at the University Arizona and Veterans Affairs Medical Center conducted a study to confirm is the combination therapy is more beneficial than just the albuterol base. The results of this study are presented in **Figure 5 (5)**.



Percentage of change in adjusted mean forced expiratory volume in 1 second from test-day baseline.

Percentage of patients in each treatment group demonstrating a 15% increase in forced expiratory volume in 1 second from test-day baseline.

Figure 5: A Comparison of Duoneb (Combivent) to plain Albuterol. Adapted from "For COPD a combination of ipratropium bromide and albuterol sulfate is more effective than albuterol base," by Campbell, S. (1999). Archives of internal medicine, 159(2), 156-160. (5)

The resulting data shows that patients taking combined therapy had a higher percentage change in FEV than those with just plain albuterol. In addition, a higher percentage of patients in the sample size had an increase of at least 15% in FEV on the combined therapy versus the albuterol. The medications were tested after 15 minutes of the dose and compared to baseline levels at 0 minutes. In addition to the increase in FEV, there were no increases in adverse side effects or worsening of pre-existing conditions. Dr. Campbell's article states,

> "However, that combination therapy did not result in an increase in adverse effects. In fact, the overall incidence of adverse events was lower in the combination therapy group (25.4%) than in the albuterol group (33.3%). Nor was combination therapy associated with worsening of adverse effects: only 14.1% of patients using the combination aerosol reported moderate to severe adverse events, compared with 22.2% in the albuterol group" (5).

These results show that treating COVID patients with these medications would be best done using the combination therapy to promote the maximum opening of the airways with as few side effects as possible. All these nebulizer medications have additional benefits when treating COVID patients since they are dosed using a nebulizer.

3.4 Benefits of Nebulizer Delivery Versus Inhalers

While these medications are commonly delivered via a nebulizer, they also come in metered dose inhaler (MDI) forms. There are also commonly prescribed as the delivery method for the medications for ill patients with other lung diseases. However, research indicates that the nebulizer has a more effective delivery method than that of MDIs. These devices release an aerosol dose when pressed which is pulled into the patent's lungs on an inbreath. This is very similar to the delivery method to the nebulizer but has some slight drawbacks. A study from Hope Hospital in Salford UK followed patients with airway obstructions over a five-year period to see the long-term effects of using home nebulizers with the previously mentioned medications to study these patients' spirometry as well as a nebulizers comparative benefit to MDIs. The

results of O'Driscoll and Bernstein indicated that patients taking the medications through nebulized methods had FEV levels higher above baseline after the study than those that used the MDI. In addition, the mortality rate amongst the nebulizer patients was slightly lower than that of the MDI patients (14). Part of this can be attributed to the need for a hand-to-breath coordination for the treatment. Some patients can struggle with taking an inbreath at the same time the dose is released from the chamber. This can lead to a decrease in the administered concentration of the drug to the patient's airways. Given that a nebulizer produces a continuous stream of aerosol, the patient can breathe normally without the need for coordination. In addition, more medication types can be delivered via nebulizer than with traditional MDIs (1). However, since aerosol is the primary method for SARS-CoV-2 transmission, it is important to understand how to safely administer nebulized medications to infected patients without causing unnecessary viral spread.

3.5 Nebulizers and Aerosol Safety

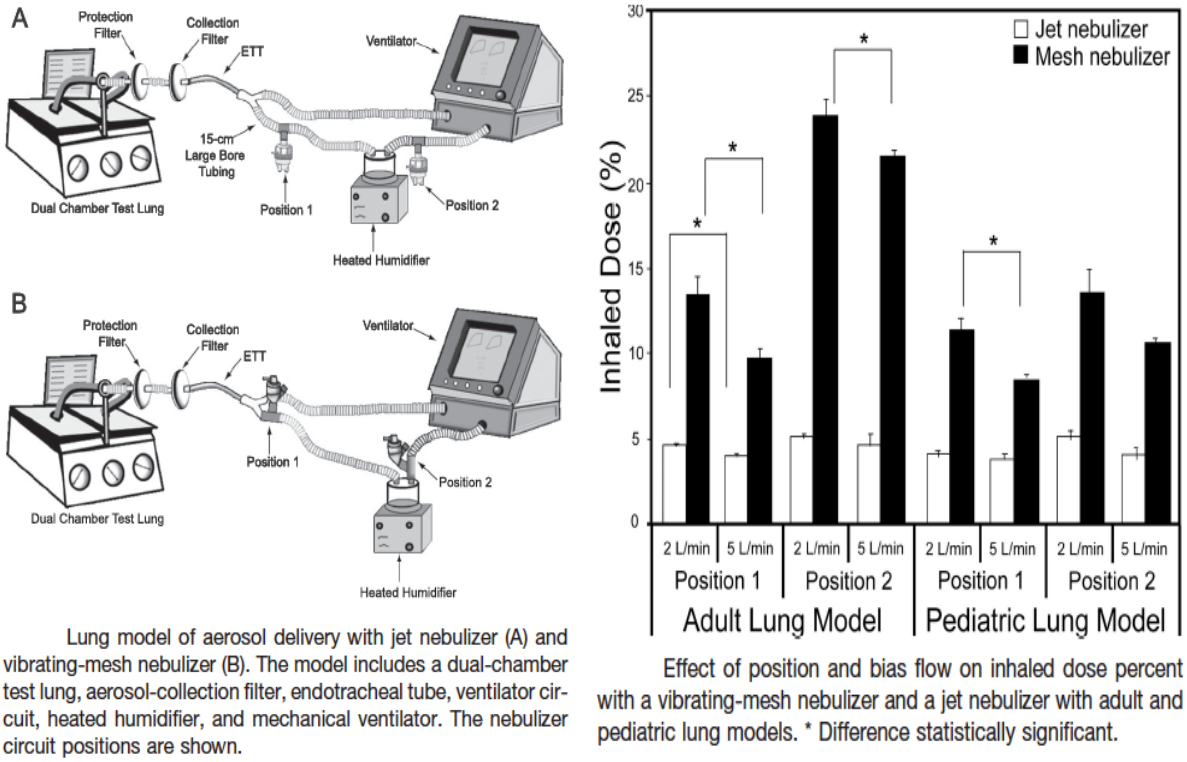
Many are concerned with the potential spread of the virus via aerosol. This brings forth the concern for safety measures in the treatment of COVID patients with nebulizer medications. There is a significant aerosol release into the ambient environment when using a typical Jetstream nebulizer to administer the patients medicine. A journal article in *Respiratory Medicine* by Dr. Arzu Ari notes that, "Although conventional jet nebulizers are commonly used to deliver aerosolized medications, they may also spew 2/3 of the emitted aerosol into the ambient environment. In this case, healthcare providers are exposed not only the inhaled medications but also to the droplets from the patient's airways and lungs" (1). However, there are ways to combat and protect both healthcare workers and other individuals from the risk of

viral transmission. In the same article Dr. Ari goes on to mention that manufacturers of nebulizer kits have developed filters to be placed on the exhalation port of the mouth pieces to cut down on this aerosol release. The article claims that, “While the placement of a filter to the nebulizer was 93% effective in capturing exhaled aerosol droplets and will reduce second hand exposure of aerosol medication to health care professionals, the efficiency of these filters in preventing the transmission and the magnitude of the risk acquiring coronavirus through filtered nebulizers are not fully known” (1). This is a very stark contradiction to the idea that exhaled aerosols can pose a threat to healthcare providers. It was also noted that mesh nebulizers with filters were even more effective at preventing the aerosol spread since they do not have a strong gaseous flow in their nebulization process. It is clear that manufacturers’ filters are highly efficient at preventing this undesired aerosol spread for infected patients in healthcare settings. For most cases, it would be even safer to send the patient home with a home nebulizer. Not only does this prevent the spread of the virus in the healthcare setting, but if a patient is given the nebulizer and medications as a preventative measure, the patient would most likely never enter a healthcare setting to even introduce potential secondhand aerosols to healthcare workers. If nebulizer medications were prescribed at the start of a patients COVID diagnosis, far fewer individuals would have severe symptoms and require hospital visits. In addition, patients that are already experiencing extreme symptoms such as ARDS would still be able to receive treatment if they were placed on a ventilator.

3.6 Nebulizers and Ventilator Patients

Many patients that develop extreme infections of SARS-CoV-2 tend to develop worse symptoms. The lung system can develop ARDS and leave patients with no capability to breathe.

This requires patients to be placed on ventilators. The use of nebulizers attached to patient ventilators can help these patients to decrease fluid and mucus production as well as de-inflame the airways. The location of the nebulizer in the ventilator circuit, as well as the type of nebulizer affects the efficiency of the medication delivery. A study was conducted using a mechanical human lung with both pediatric and adult settings. A circuit with a nebulizer was attached to the ventilator machine at different locations with different types of nebulizers. The circuit was tested with both Jetstream and mesh nebulizer types. The study was conducted by Ari et al. from the division of Respiratory Therapy at the School of Health Professions at Georgia State University. In their study they found that mesh nebulizers at the position placed before a heated nebulizer had the most effective deposition of the dose distal to the endotracheal tube. The results of their experiment are listed in **Figure 6 (2)**.



Lung model of aerosol delivery with jet nebulizer (A) and vibrating-mesh nebulizer (B). The model includes a dual-chamber test lung, aerosol-collection filter, endotracheal tube, ventilator circuit, heated humidifier, and mechanical ventilator. The nebulizer circuit positions are shown.

Effect of position and bias flow on inhaled dose percent with a vibrating-mesh nebulizer and a jet nebulizer with adult and pediatric lung models. * Difference statistically significant.

Figure 6: Study Results of Nebulizer Type and Position with Ventilator Patients. Adapted from "Influence of nebulizer type, position, and bias flow on aerosol drug delivery in simulated pediatric and adult lung models during mechanical ventilation," by Ari, A., Atalay, O. T., Harwood, R., Sheard, M. M., Aljamhan, E. A., & Fink, J. B. (2010). Respiratory care, 55(7), 845-851. (2)

This shows that nebulizer medications can be effectively delivered to a ventilator patient with COVID-19 to help alleviate symptoms. The medication used in this experiment was albuterol sulfate and they concluded that using a mesh nebulizer at position two would possibly improve bronchodilatation (2). They found that it benefits to place the nebulizer at position 2 because it also takes the weight of the machine away from the patient airway which is better for smaller patients. A faster speed of aerosol flow through the circuit showed that less would be taken into the patient's airways, which is why a mesh nebulizer, which has no additional gaseous flow, helps to increase the amount of medication delivered to the patient's lungs. The previously

mentioned article by Arzu Ari also stated, “Mesh nebulizers can stay in-line for up to 28 days, and reservoir design allows adding medication without requiring the ventilator circuit to be broken for aerosol drug delivery. Unlike the jet nebulizer, the medication reservoir of mesh nebulizers is isolated from the breathing circuit that eliminates the nebulization of contaminated fluids” (1). This shows a higher level of safety for practitioners taking care of patients in intensive care units as they will have minimal exposure to secondary aerosols from infected patients, although filters that are used with nebulizers are also effective when in circuit. That article mentioned that the amount of drug deposited at the exhaust port was greater than 160-fold higher without the expiratory filter than with the filter in place (1). Nebulizer medications are not the only proposed treatments for COVID-19 patients. However, some of these other treatment options have some drawbacks that make them poor therapy options.

3.7 Alternate Therapy Options and Their Drawbacks

As stated previously, there is no conclusive treatment option for COVID-19. However, many suggestions have been made as to what mediations can and will improve symptoms. One common treatment option suggested is the use of angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs). These medications are commonly used in patients that have cardiovascular conditions such as hypertension and heart failure (6). The idea is that these medications will lower the body’s production of AngII and help alleviate inflammation in tissue. The problem with these medications is that they can actually increase the amount of ACEII receptors in the cardiopulmonary circuit. This gives SARS-CoV-2 more target proteins to bind to and more opportunity to enter host cells. Dr. James Diaz, a Professor of Anesthesiology at Louisiana State University states, “Intravenous infusions of ACEIs and ARBs

in experimental animals increase the numbers of ACEII receptors in the cardiopulmonary circulation. Patients taking ACEIs or ARBs chronically for cardiovascular diseases are assumed to have increased numbers of ACE2 receptors throughout their cardiopulmonary circulations as observed in experimental animal models" (6). In his hypothesis he also goes on to mention that laboratory studies from China have showed that patients with pre-existing conditions treated with ACEIs and ARBs had more chronic symptoms with their SARS-CoV-2 infections. Another treatment option that has been suggested is the use of beta-adrenergic blockers as a possible treatment for a SARS-CoV-2 infection. These drugs work to halt renin production and inhibit the RAS system cycle. This method of treatment shows promising results as a preventative treatment, as it lowers the amount of ACEII receptors in the pulmonary system and gives less opportunity for spike protein binding (16). However, it does also shut down and eliminate the ACEII function of breaking down target angiotensin peptides. This means patients that are already experiencing inflammatory symptoms may have a longer timeframe being inflamed as a key component of de-inflammation has been removed. An article in *BioEssays* written by Natesan Vansanthakumar does show that this treatment type does decrease multiple second messenger inflammatory molecules such as TNF-α, but fails to touch on the loss of benefit from deactivating ACEII based de-inflammation. While no method of treatment will be completely perfect, we need further investigation and testing in all posed treatment options to gain the best understanding of each one's benefit.

3.8 Patients Already Being Treated with Nebulizer Medications

These target medications have already shown to be effective in treating certain airway diseases. Conditions like COPD and asthma also are currently treated with these medications and

these patients can also contract a SARS-CoV-2 infection. Interestingly, patients already being treated with one of these medication types for a pre-existing airway condition appear to be encouraged to continue taking these regimens. Alexzandra Hughes-Visentin & Athea B. M. Paul state that, “However, similar to other coronaviruses, it is also hypothesized that SARS- Cov-2 will precipitate asthma exacerbation. Therefore, further investigation into the immunopathological mechanism still needs to be elucidated to determine risk of severe exacerbations in asthmatic patients. It is for these reasons among others that it is recommended that asthmatic patients continue their maintenance medications throughout the pandemic” (8). They go on to also mention that combination therapies of long-acting beta-agonist drugs reduce SARS-CoV-2 replication and cytokine production, medications that are already used to treat asthma patients. Asthmatic patients were also shown to have less severe symptoms of COVID contrary to popular beliefs on viral induced exacerbation. “The respiratory epithelial cells in patients with asthma have decreased gene expression for ACE2 receptors and therefore may be protective against COVID-19 infection”, Alexzandra Hughes-Visentin & Athea B. M. Paul note (8). Both the existing airway condition as well as the nebulized medications themselves potential serve to help ease the severity of infection. This shows that while further investigation needs to be done, lesser symptom results of COVID-19 when patients are previously medicated on a nebulizer cannot be ruled out.

Chapter 4: Local Physician Input

To gain a better understanding of this treatment option for COVID patients, surveys were given to physicians in the Murray Kentucky area. The survey consisted of questions to gauge if physicians felt as though nebulizer medications would be an effective treatment. Other questions in the survey focused on the physicians' experience as healthcare professionals and their experience with COVID patients. All responses were to be anonymously aggregate in the following section. Approximately 40 surveys were given out over the course of two months. A total of one survey was collected given the unresponsiveness of physicians. Intensive efforts were made to collect the data, including in office visits and numerous phone calls to nursing staff. The one physician signed a reviewed IRB consent form and understood that their responses would be anonymously aggregated in the thesis and their name and practice would only be mentioned at their given consent. The singular survey response was not elected to be included since it did not represent an accurate sample size of local physician opinions. The survey questions are included below for reference to the material presented.

4.1 Physician Survey Questions

Introduction and Personal Question Responses

Practice Type:

Number of Years as a Practicing HealthCare provider:

Any Specialties or Additional Focuses (ex. Pulmonology):

Would you be willing to have your name or practice mentioned in the content of this Thesis?

Circle: YES NO

Have you practiced mainly in rural areas or larger urban populations during your career?

Circle: Rural (Small Towns) Urban

Please briefly describe the different settings you have worked in and which one you worked in Primarily over the course of this Pandemic:

True and False Question Responses

1. My staff and I have treated or cared for COVID patients before. True False

2. My staff and I treat and test COVID patients on a daily basis. True False

3. I recommend the CDC quarantine guidelines to my positive patients. True False

4. I feel as though prescription medicines help with a COVID infection. True False

5. I have treated a large amount (>100) of positive patients. True False

6. If I write an RX for a positive patient, my first choice is an inhaler or a nebulized treatment.

True False

7. I am familiar with the mechanism of action of short acting muscarinic receptor antagonists (SAMA) and short acting beta receptor agonists (SABA) drugs within the human lungs.

True False

8. I think that SAMA and SABA drugs (nebulized or inhaled) would be effective treatments for COVID Patients.

True False

9. The patients I have seen that already used these types of medications have had a less severe response to covid, from a respiratory perspective.

True False

10. Opening and clearing the lungs is the best way to keep patients off ventilators.

True False

11. I am concerned that using nebulized methods or inhalers could increase the spread of COVID via air particles.

True False

12. Our healthcare system has the resources to adequately treat COVID patients.

True False

Open Response Question Responses

What are some other treatment options that you have seen be effective for COVID patients?

Do you think that the inflammation cause by COVID is most detrimental side effect? Why?

Acute Respiratory Distress Syndrome (ARDS) usually results in patients being put on ventilators in the ICU. Please describe to the best of your ability the key issues when a patient develops ARDS.

Depending on your previous answer in the True/False section, please describe why or why not a SAMA/SABA or other bronchodilator would work for a COVID infection.

Do you feel as though we will see an "end" to this pandemic, or do you think this virus will eventually become a very common illness?

Should we focus our efforts to care for COVID patients on treating their symptoms or on finding a cure for this illness?

All responses will be aggregated anonymously in the appendix of the thesis. If I included a quotation from your responses in the body of the work, may I cite this quote to you?

YES NO

Conclusion

Even without successful input from local area physicians, the principal use of nebulizer medications for COVID-19 treatment still remains viable. While further investigation into the effects of these drugs on COVID patients' needs to be made, biological pathways point to the ability to treat patients' symptoms with these medications. There is also an importance placed on the safety aspects of delivering medications via nebulizer. We have seen that filters and home use can help prevent unnecessary exposure to aerosols. With these medications on the market already, they will be easy to access and effective for patient care. Nebulizer medications can also be administered to COVID patients at any point in their diagnosis, even up to when ventilation methods are administered. Overall, this treatment option needs to be considered for patient in all healthcare settings and further studied for efficacy.

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