THE MOLECULAR MECHANISM BEHIND THE PATHOGENIC ACTION OF THE SARS-COV-2 VIRUS, THE ROLE OF THE ACE2 RECEPTORS IN THE CREATION OF SURFACTANT AND IN THE PROTECTION OF LARGE FUNCTIONAL SURFACES

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Abstract: COVID-19 is caused by the SARS-CoV-2 virus. SARS-CoV-2 is different from other corona viruses due to the fact that it can intensely bind to the ACE2 receptor. Expression of ACE2 receptors is especially characteristic for the following cells: alveolar type 2 cells, endothelial cells of both small and large arteries, and the smooth muscle cells of the arteries, enterocytes of the small intestine, Leydig and Sertoli cells, proximal cells of the renal tubules and intestine cells. A common characteristic of these organs, tissues and cells, which have a high ACE2 expression, is that they have a "large functional surface". ACE2 receptor is critical in maintaining the integrity and stability of these so called "large functional surfaces". Production of surfactant in order for the ACE2 receptor to realize its protective function. Surfactants role in the stabilization and immunoprotection of large functional surfaces. People with a lower number of ACE2 receptors (the obese, elderly, people with comorbidities, males) are more susceptible to the virus occupying all avalaible ACE2 receptors and blocking the production of surfactant to such a degree that the antigens of ''large functional surfaces" become visible to the immune system and cause a massive inflammatory response in the COVID-19 cases. The molecular mechanism of the pathological action of the SARS-CoV-2 virus as explained from the aspect of ACE2 binding and the subsequent inhibition of surfactant production.
Keywords: Covid-19, SARS-CoV-2, ACE2, surfactant, large funcional surface

Sensitive groups and comorbidities

The lethality of COVID-19 is in correlation with the age of the patient, the lethality among younger individuals is much lower compared to the relatively higher lethality among the elderly (1, 2, 3). People above the age of 65, who also have comorbidities (hypertension, diabetes, obesity, kidney and cardiovascular issues), are especially at risk. Most people who pass away due to COVID-19 have had underlying health issues (hypertension, diabetes and heart disease) (4). Based on the data from the month of March in the United States 89% of those hospitalized due to COVID-19 have had underlying issues (5). The Italian Superiore de Sanita institute reported that of the patients that died due to COVID-19, the ones that had medical documents, 96.1% had at least one comorbidity and the average amount of comorbidities per patient was 3 to 4 (6). Based on this report the most common underlying issues were: hypertension (66% of cases), type 2 diabetes (29.8% cases), heart disease (27.6% cases), atrial fibrillation (23.1% cases) and chronic kidney disease (20.2% cases). Hypertension is closely linked with a more severe case of COVID-19 and is one of the most common underlying issues in patients with severe pneumonia due to COVID-19 (7, 8, 9, 10, 11). Obese is also linked with more severe COVID-19 cases (12, 13, 14). Also there is a difference in the amount of severe cases between genders, men are more likely to suffer from severe COVID-19. Early epidemiological data from China and Italy have shown a higher death rate in men (15, 16, 17). In addition, there is the fact that in Europe 57% of those infected with SARS-CoV-2 were men while 72% of fatal cases were also men (18).

Reduced expression of ACE2 receptor in sensitive groups

Scientists have discovered that ACE2 expression in 30 different tissues, collected from thousands of different patients, drops significantly after the age of 60 (19). This correlates perfectly with what is seen in the field, children and the young often have mild symptoms while the elderly have overall more severe symptoms due to COVID-19. The same scientists that had discovered the reduced expression of ACE2 receptors in the elderly also discovered that diabetics, especially those with type 2 diabetes, also have reduced ACE2 expression. Also, a reduction in ACE2 expression in the glomeruli and tubules of the nephrons was found in those affected by type 2 diabetes and chronic kidney disease (20).

ACE2 expression is high in the endothelium of the heart and kidney. Expression of ACE2 is significantly reduced in spontaneously hypertensive rats. What we can conclude from this is the fact that ACE2 expression is reduced in hypertension (21).

In animal models it has been shown that ACE2 expression drops off with age and is also lower in males compared to females. This gives us an explanation as to why we see much more severe COVID-19 cases and COVID-19 related complications in older males (22, 23).

As we have mentioned above people with hypertension are especially at risk of developing severe issues due to COVID-19, due to the fact that in hypertension the expression of ACE2 receptors is below a certain threshold (24).

Chaudhry F, Lavandero S, Xie X, et al. have created a great theoretical model which illustrates that a reduction in ACE2 expression below a certain threshold is needed in order for severe lung damage to occur and potential lung fibrosis to begin (25) (Figure1.).

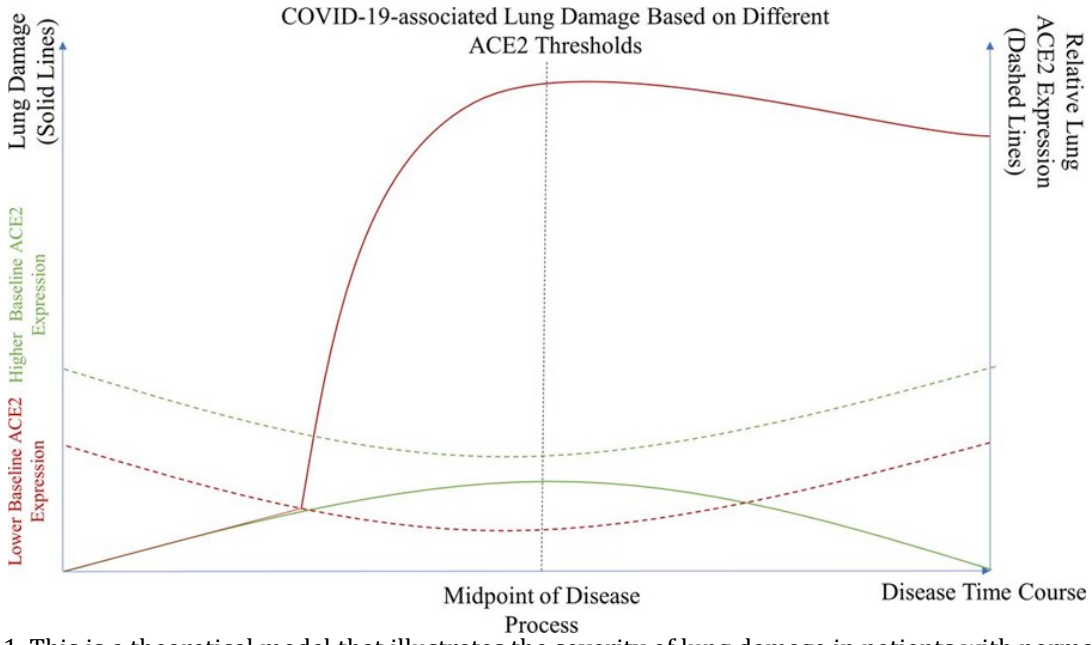

Figure 1. This is a theoretical model that illustrates the severity of lung damage in patients with normal ACE2 expression (full green line) as well as in patient with reduced ACE2 expression that is mainly associated with comorbidities or risk factors (full red line). Patient with a higher base level of ACE2 expression never fall below the critical level of ACE2 expression (green dotted line) and are far less likely to suffer from severe lung damage. On the other hand patients with a lower base level of ACE2 expression (red dotted line) are far more likely to suffer from severe lung damage.

The role of the ACE2 receptor from the aspect of the pathological mechanism behind the action of the SARS-CoV-2 virus

When it comes to COVID-19 the one thing in common with all organs affected by this disease is the fact that these organs have “large functional surfaces” that need to be protected from an unregulated immune response. The lungs are the best example for an organ such as this (the alveoli and bronchi have a large functional surface). The ACE2 receptors are primarily expressed on the club cells of the bronchiole and on type 2 pneumocytes of the alveoli. Both of these cells protect the lungs and help prevent the onset of Acute

Respiratory Distress Syndrome (ARDS). Club cells secrete a solution that is similar to surfactant as well as other proteins which protect the airways from severe inflammatory response by the immune system. While type 2 pneumocytes defend the alveoli by secreting and recycling the surfactant which is necessary to maintain normal surface tension of the alveoli (26). The ACE2 receptors are far more expressed on the cells of the lower airways than on the cells of the upper airways (27).

Single cell RNA sequencing has revealed that the ACE2 receptor is especially expressed on the surface of type 2 pneumocytes in the alveoli. But, the ACE2 receptor is also expressed in organs that have a "large functional surface", such as: the kidneys (proximal tubule cells), myocardium cells, the enterocytes which line the ileum, the cells in the esophagus and the uroepithelial cells of the bladder (28). From the facts above we can conclude that the ACE2 receptor has a protective role in the organs that have "large functional surfaces", the ACE2 receptor protects these surfaces from an inflammatory response by the immune system.

The fact that ARDS occurs in severe COVID-19 cases only supports the fact that the ACE2 receptor has a protective role. Lung biopsies have unveiled severe inflammation and edema, which directly corresponds with animal models in which lowered ACE2 expression is associated with severe lung damage (29, 30). Those who had severe H5N1 influence infections had similar findings (overreaction of the immune system and a large amount of cytokine production) (31).

The molecular function of the ACE2 receptor on the cell

ACE2 (Angiotensin converting enzyme 2) belongs to a family of angiotensin converting enzymes also known as dipeptidyl carboxypeptidase. Besides the functions mentioned above ACE2 also converts angiotensin 1 to angiotensin 1-9 and it also converts the vasoconstrictive angiotensin 2 into the vasodilative angiotensin 1-7. ACE 2 is highly important in regulating the renin-angiotensin-aldosterone which in itself is necessary in maintaining optimal blood volume, blood pressure and as such is crucial in the normal functioning of the cardiovascular system (32, 33).

Furthermore the ACE2 receptor also removes the C terminal remains of many vasoactive peptides such as: neurotensin, kinetenin and des-Arg bradykinin (34, 35). Besides these vasoactive peptides ACE2 also catalyzes the breakdown of casomorphins, dynophrin A and apelins (35, 36) .The ACE2 receptor is also very important for the transfer of neutral amino acids through the gut lining (37).

Expression of ACE2 receptors is especially characteristic for the following cells:

-alveolar type 2 cells (38, 39, 40)
-endothelial cells of both small and large arteries, and the smooth muscle cells of the arteries (41)
-enterocytes of the small intestine, Leydig and Sertoli cells (41)
-proximal cells of the renal tubules and intestine cells (37)
-heart, kidney, testicle, and the gastrointestinal tract (34, 42, 43, 44, 45, 46)

A common characteristic of these organs, tissues and cells, which have a high ACE2 expression, is that they have a "large functional surface". These are large cellular surfaces which are critical for the normal function of the organs mentioned above. From this point of a view we can conclude that the ACE2 receptor is critical in maintaining the integrity and stability of these so called "large functional surfaces". We can also conclude that the ACE2 receptor is important in the production of surfactant in order for the ACE2 receptor to realize its protective function.

The lungs have an especially large functional surface. In the lungs the ACE2 receptor is located on the surface of alveolar type 2 cells (AE2). It is a known fact that these cells are highly important in the production of surfactant. Also it is known that the main role of surfactant is to reduce the surface tension of alveoli and prevent their collapse. Our findings however suggest that the cells that produce surfactant, also have an immunomodulatory role (47).

The connection between acute lung injury, surfactant production, the ACE2 receptor and an overreactive inflammatory response is best displayed in experiments done on rats. Reduced ACE2 expression is directly correlated with reduced production of surfactant (48).

Many authors have noted and recognized the important role that surfactant and the ACE2 receptor have in protecting the lungs, as well as the use of naturally and synthetically produced surfactant in the treatment of COVID-19 patients (49).

The SARS-CoV-2 virus inhibits the production of surfactant by firmly binding with the ACE2 receptor

As we have mentioned above the SARS-CoV-2 virus differs from other SARS viruses by having a novel mutation in the S protein which grants it the ability to firmly bind to the ACE2 receptor in order to enter the targeted cell. By firmly binding to the ACE2 receptor the virus has gained another mechanism by which it can damage the host, and that mechanism is the inactivation of the ACE2 receptor via intense binding with said receptor. We can confirm this by observing people who inherited dysfunctional ACE2 receptors. These people commonly suffer from Severe Acute Respiratory Distress syndrome which mirrors severe COVID-19 cases. Besides COVDI-19, dysfunctional ACE2 receptors are also associated with: hypertension, kidney disease, myocardial infarction, type 2 diabetes and blood vessel disorders (56). These are all comorbidities that are associated with severe COVID-19 cases, and due to these factors it is necessary that we begin researching the role that the ACE2 receptor has in producing surfactant in those with comorbidities.

The SARS-CoV-2 virus can inhibit the production of surfactant by firmly binding with the ACE2 receptor.

The over reactive immune inflammatory response in the lungs seen during SARS and ARDS that is caused by a lowered production of surfactant is in itself sometimes caused due to a mutation in the following genes necessary for surfactant production: SFTPA, SFTPB, SFTBC, SFTBD, SFTA2, SFTA3. Mutations in these genes cause similar consequences to those seen in ACE2 receptor inhibition via the SARS-CoV-2 virus. The consequences we are talking about are Respiratory Distress Syndrome in Premature infants, Pulmonary Fibrosis and Interstitial pneumonia (51, 52, 53, 54, 55).

The conclusion from the information mentioned above is that no matter the cause of ACE2 receptor inhibition (via the SARS-CoV-2 virus or via mutation) or surfactant production inhibition the end result is the same when it comes to lung damage. Also other organs are affected.

We can safely say that in order for the virus to achieve its pathological potential on the molecular level it is necessary for it to inhibit surfactant production and to disrupt the surface tension of the alveoli.

Surfactants role in the stabilization and immunoprotection of large functional surfaces

When it comes to so called “large functional surfaces” the role of surfactant is two-fold. Its first role is in the reduction of surface tension and its second role is in the protection of these surfaces from the immune system.

These are two very important and broad functions. Surfactant has to ensure that these large surfaces remain functional while at the same time it has to protect these large surfaces and their antigen specificities from systemic immunity.. Surfactant has a very gentile molecular structure and as such is easily changed, which can have systemic consequences. As mentioned above, organs and tissues which have and need large functioning surfaces are: small blood vessels, the alveoli of the lungs, the tubules and nephrons of the kidneys, the GI tract and even blood platelets.

The major piece of evidence in the case that the SARS-CoV-2 virus affects the lungs via blocking the ACE2 receptor and subsequent surfactant production are the groups most affected by COVID-19. The groups mostly affected are: people with hypertension, cardiovascular patients, obstructive lung disease patients, people with chronic kidney disease, the elderly (due to reduced elasticity in the blood vessels and airways). All of the diseases mentioned in this paragraph have one thing in common and that is the fact that the large functional surfaces are damaged and not functioning optimally.

Surfactant is important on all large functioning surfaces. This is especially the case when it comes to the lungs (57, 58), where large surfaces are needed to facilitate the interaction between the air that is inhaled and the circulating blood (59, 60). These surfaces are vulnerable to and needs to be protected from an inflammatory immune response (61, 62). A local inflammatory response here can quickly escalate to a large systemic inflammatory response. When such an inflammatory response escalates it quickly evolves into SARS.

The molecular mechanism of the pathological action of the SARS-CoV-2 virus as explained from the aspect of ACE2 binding and the subsequent inhibition of surfactant production

After infection by the SARS-CoV-2 virus a literal race against the clock begins between the rate of viral replication and the immune system response to the virus. This is a race against the clock in which the goal is to remain at a normal level of surfactant production.

The virus needs the ACE2 receptor in order to enter the cell. When the virus binds to the ACE2 receptor, the production of surfactant is inhibited. The more viral particles there bind to this receptor the less surfactant is synthetized.

If the infected person has a large amount of ACE2 receptors then the amount of viral particles needed in order to reduce this number below a critical threshold (as mentioned in the beginning of this article) is also

increased. This gives the immune system enough time to effectively counteract the SARS-CoV-2 virus. That is why people that are young, healthy, physically active and females are much more resistant to COVID-19, due to the increased amount of ACE2 receptor (the full green line mentioned in the beginning of this article).

People with a lower number of ACE2 receptors (the obese, elderly, people with comorbidities, males) are more susceptible to the virus occupying all avalaible ACE2 receptors and blocking the production of surfactant to such a degree that the antigens of „large functional surfaces“ become visible to the immune system and cause a massive inflammatory response that we see in ARDS and SARS (the red line in the picture in the begining of this article). The lungs are usually the first site of this inflammatory response due to the fact that surfactant is critical for their normal function. This is basically a kind of inflammatory autoimmune response. This is confirmed by the fact that immunosuppressive therapy, which is normally used to treat autoimmune disorders, has thus far been highly effective in the treatment of severe COVID-19 cases.

Take into consideration the lowered production of surfactant due to ACE2 receptor inactivation. By implementing simple lifestyle changes such as: physical activity and diet, the stability of surfactant production can be improved. Also the role which vitamin D has in the increase of ACE2 receptor expression, especially in sensitive groups, must be taken into consideration when it comes to using vitamin D supplementation as a preventative and protective measure in sensitive groups. The knowledge gained by studying surfactant should be used in the treatment of chronic diseases such as: hypertension, cardiovascular diseases and others.

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