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Hypothesis: Advanced Biotechnological Treatment Approaches Against Sars-COV-2 (COVID-19)

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Abstract:- The new type of coronavirus, called COVID-19, which began to spread all over the world, caused a pandemic. According to the 24th March data, there is no cure for this viral acute respiratory disease, which caused 17,147 deaths. Healthcare professionals use medications used in previous coronavirus-induced diseases to relieve symptoms in treatment. Researchers, on the other hand, evaluate the comparative effects of these drugs and try to find a new drug or vaccine. We are publishing a study that biotechnological combinations of used and non-toxic effective drugs can be an effective approach to the disease.

I. INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel infectious disease, was firstly found in Wuhan, China, in December 2019 (1). Up to now, over 82,000 cases of SARS-CoV-2 (COVID-19) were reported, > 2800 deaths (2). SARS-CoV-2 has been sequenced and it was determined as a result of phylogenetic analysis that SARS-CoV-2 is of bat origin. There are many similarities of new type COVID19 (SARS-CoV-2) with the original SARS-CoV. Xu et al. proved that the spike proteins of COVID19 (SARS-CoV-2) and original SARS-CoV are similar. Also, they found COVID19 (SARS-CoV-2) and SARS-CoV spike proteins display 76.5% identity in amino acid sequences. (3)

- *SARS CoV-2 (COVID-19) Treatment Approaches with Angiotensin-Converting Enzyme 2 and Camostat Mesylate*

Various treatment approaches, such as the spike protein-based vaccine for ACE2-mediated COVID-19, inhibition of transmembrane protease serine 2 (TMPRSS2) activity, blocking the ACE2 receptor, administration of the soluble form of ACE2, are also described as shown below (4).

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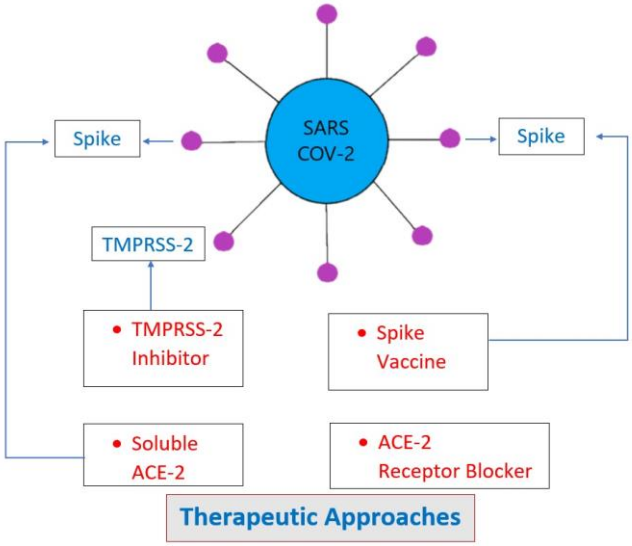

Fig 1:- Therapeutic Approaches for SARS-CoV2

Angiotensin converting enzyme 2 (ACE2) is a well-known monocarboxypeptidase that is found in the human body to separate several peptides in various substrates. ACE2 is in the lower respiratory tract of humans, has been previously identified as the cell receptor for SARS-CoV. This receptor has the task of regulating both viruses that spread among different species and human-to-human transmission (5). ACE2 receptors serve as binding sites for the spike (S) proteins on the exterior surfaces of both original SARS-CoV and SARS-CoV-2 (COVID19) that cause the severe acute respiratory syndrome (6).

Camostat mesylate, a TMPRSS2 inhibitor, is a potential therapeutic agent for SARS-CoV, MERS-CoV and now SARS-CoV-2(COVID19). The viral spread and pathogenesis of SARS-CoV infection in a pathogenic mouse model has been shown to be effectively blocked by the camostat. Since camostat is in clinical use for the treatment of chronic pancreatitis, it represents a potentially safe and effective therapeutic option (7).

TMPRSS2 activating viral spike glycoproteins that proteolytically cut and facilitate the fusion of the virus cell into the host cell; it allows for the refolding and energy release required to create stable virus-cell bonds and membrane fusion (8)

II. METHOD OF ADMINISTRATION OF BIOTECHNOLOGICALLY PREPARED MEDICINE

After the discovery of the complex sequence of the human genome, detailed study of disease-related genes and microstructures made it possible to produce highly reliable drugs with biotechnology. With the use of biotechnology in the pharmaceutical field, drugs with high selectivity and effectiveness were introduced to the use of medicine. (A review article Biotechnology Applications in Medicine.). In this part of our article, we will briefly explain the type of biotechnological drug deemed suitable for COVID-19 and how it should be applied.

➢ *Aerosols*

Low dose usage and decrease of side effects is the most important advantage of this treatment method in the transportation of pharmaceutical compounds by inhalation (9). Generally, parenterally administered drugs such as vaccines, antiviral compounds, hormones can also be administered as an inhaler. It is an important advantage that it is easier to apply than parenteral administration and that no sterilization is required during the preparation of the formulation. It is important to protect the active substance from enzymatic destruction in the gastrointestinal tract and to prevent the first pass effect in the passage through the liver and because they pass slowly and slowly into the systemic circulation, their side effects on the heart, other organs are reduced and their selectivity on the bronchi is increased. Since the drugs reach the bronchial smooth muscles at a higher concentration compared to systemic application, they have a maximal effect. Because their local metabolism is slow in the bronchial wall, their effects are longer lasting. By using the metered dose valve, the dose can be adjusted according to personal need. If the drug shows a variable pharmacokinetic behavior in oral or parenteral use, the aerosol drug form may be considered as an appropriate alternative (10) (11) (12).

➢ *Use of Nanoparticles*

Three-dimensional and cross-linking hydrogels are hydrophilic polymers. Sodium alginate is one of the most commonly used natural polysaccharides. Sodium alginate is a non-toxic, biocompatible, and biodegradable polysaccharide with several unique physicochemical properties. Due to these properties, sodium alginate is a unique polysaccharide that can be used as a delivery tool for

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drugs. (13, 14). Therefore, we preferred to use alginate nanoparticles in our project to use against COVID-19, which has a high rate of infecting cells, due to its drug release properties in a short time.

A biocompatible cationic polymer, chitosan, have numerous functional groups for targeting ligand modification. Furthermore, chitosan coating improves the particle stability and controls drug release. Chitosan's has muco-adhesive property. Therefore, it can be exploited for trans-mucosal delivery of drugs, especially through the intrapulmonary way (15). We also added chitosan polymer to our project because it is suitable for ligand modification, has mucoadhesive properties and increases the particle stability.

Biodegradable polylactide-co-glycolide (PLGA) nanoparticles also have been used as carriers for drugs, vaccines, and nucleotides. Moreover, the US Food and Drug Administration (FDA) approved the use of PLGA polymers in parenteral microspheres, implants, and periodontal drug-delivery systems (16). PLGA is another biocompatible polymer that we aim to use in our project as it provides drug release in a longer time.

Microspheres and nanoparticles can be targeted to specific organs or tissues as a function of particle sizes. As a result of the studies carried out, the prepared microspheres, nanoparticles and nanocomposites can be prepared at acceptable production efficiencies, they are close to the sphere and are homogeneously distributed, have appropriate particle sizes and are stable, the active substance and polymer are compatible, have effective drug loading capacity and have 8 to 24 hours. It has been observed that it can be formulated with properties that can be used in the treatment of respiratory diseases in the lung, showing controlled release between the patients (17). Based on this information, we planned to use chitosan coating sodium alginate and biodegradable polylactide-co-glycolide (PLGA) nanoparticles in our study.

Hypothesis 1: Camostat Mesylate Loaded Core shell ACE2 Embedded Chitosan/Alginate Nanoparticles

In this hypothesis, we aimed to embed the camostate mesylate drug into chitosan / alginate nanoparticles that contain ACE2 receptors on the surface. We believe that such a combination can have 2 advantages for us, and we have called it as "double hit". First, when ACE2-coated nanoparticles are taken by inhalation, binding of SARS-CoV2s carrying Spike protein compatible with ACE2 will provide a virus-specific inhibition system. Even if a second Spike protein of the "nanoparticle-bound virus particle" interacts with any ACE2 present on the lung surface, it cannot be taken into the cell by endocytosis. For this reason, nanoparticle-bound virus particles that circulate outside the cell will be removed by the immune system of our body in a short time. Another effect is that when the virus-induced alginate nanoparticles start to degrade within 1 hour, the camostat mesylates will be released and the entering of the viruses into the cell will be prevented by inhibition of the TMPRSS2 proteases in the host cell.

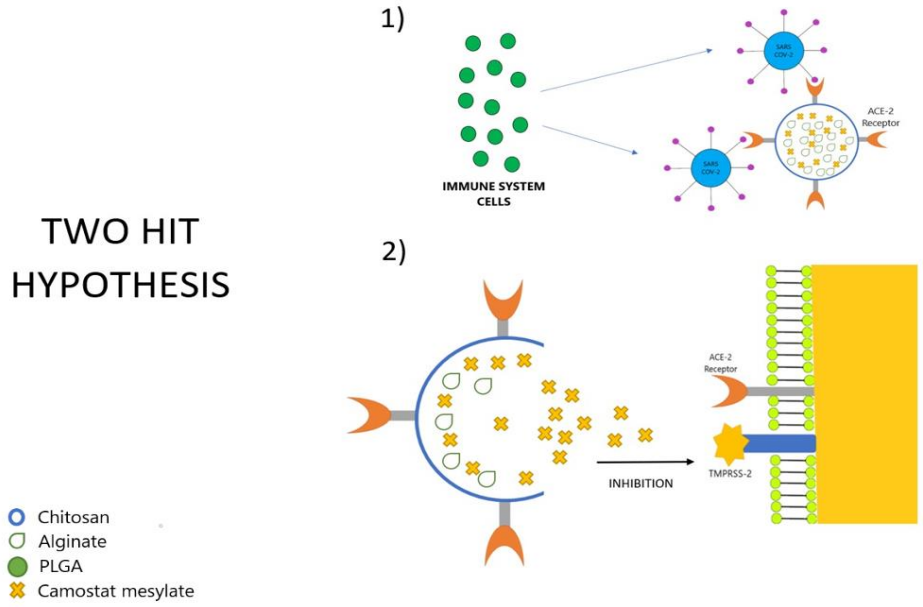

Fig 2:- Our Treatment Approach

While writing this hypothesis article, we aimed to design a 2-hit biotechnological drug;

- The SARS-CoV 2 virus contains spike glycoproteins (purple-black bars on the virus surface) that can specifically bind to the ACE-2 receptor in the host cell. The part shown on the right side represents the membrane of the host cell. ACE2 on the nanoparticle binds to the spike protein to prevent our virus to binds with ACE2

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which is on the host cell surface as mentioned before. Viruses adhering to ACE-2(on nanoparticle) will have a large form that will prevent the host cell from making endosomes. At the same time, it will be easier for the larger virus-nanoparticle compound to be seen by the immune system.

- Preventing viruses from entering the cell by releasing the Camostat in the contents of the nanoparticle even if the viruses couldn't attach to the ACE2 receptors on the nanoparticle surface via the inhibition of TMPRSS2 which causes to activate virus.

Hypothesis 2: Long-Term Effect of the Camostat with Slow-release Mechanism

In this hypothesis, the treatment mechanism operates similarly to the first hypothesis. However, in addition to camostate mesylate ACE2 coated chitosan / PLGA nanoparticles will be included in the chitosan / alginate nanoparticles. In this way, there will be 2 special benefits that the combination will provide us;

- Thanks to the alginate nanoparticles being degraded within hours, a quick effect will be achieved through the camostat inside.
- PLGA nanoparticles, which take at least 1 week to be completely degraded at the earliest stage, will be released after the chitosan / alginate has been degraded and will show its effect through a slow release of camostate in oscillating pattern for 1 week.

As explained in the introduction, alginate and chitosan nanoparticles are biocompatible and provide the controlled release for drugs, which is the crucial part of our treatment method. The addition of ACE2 receptors to these nanoparticles and the binding of virus particles to ACE2 receptors are other key points in our treatment protocol. Camostat mesylate, which has already been tested and has achieved significant results against SARS-CoV-2, is another important part of our treatment protocol.

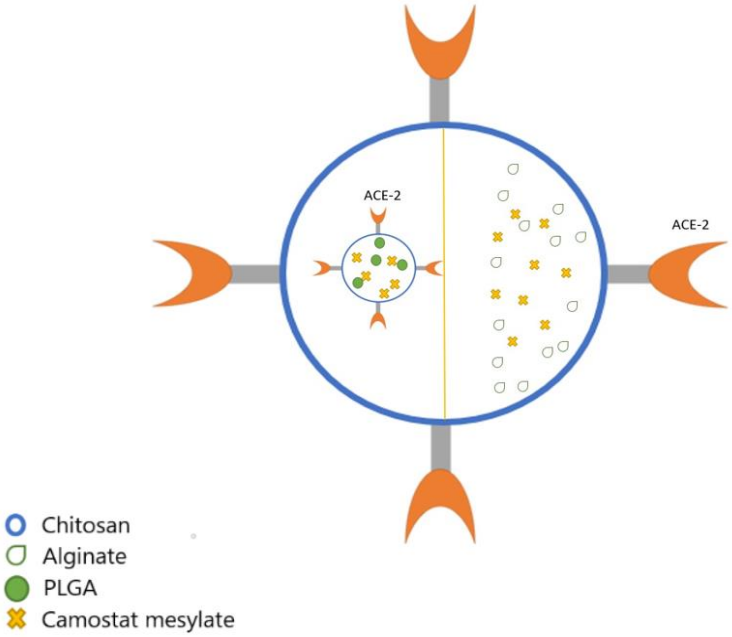

Fig 3:- Controlled Release Drug

In the second hypothesis, we add PLGA nanoparticles into the alginate nanoparticles so that we ensure slow release and ensure that the drug remains in the body for as long as the virus in the body goes out.

III. CONCLUSION

In this way, the combination of existing methods and the use of smart and advanced technological treatment protocol in combination increases the possibility of being tested on patients in a short time. When we evaluate in terms of cost, this hypothesis is one step behind compared to the previous one, since the PLGA used in our second hypothesis is more expensive. However, we believe that the treatment protocol included in our second hypothesis may be superior in terms of providing long-term and controlled treatment.

Note: Our study does not require ethics committee permission.

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