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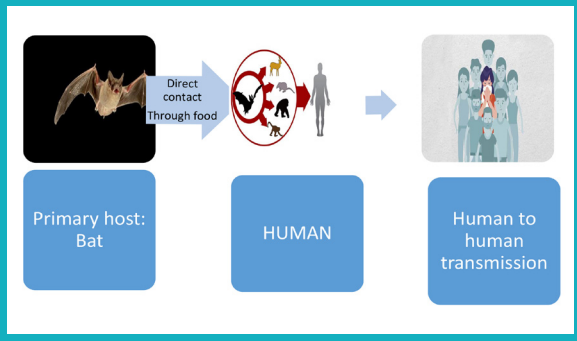
"Therapeutic Options and Potential Vaccine Studies Against Covid-19"

-Part II-



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Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992.p.1561-5.

Scientific or Technical Report: Smith P. Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX) Dept. of Health and Human Services (US). Office of Evaluation and Inspections: 1994 Oct. Report No: HHSIGOE 169200860.

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Manuscripts accepted for publication, not published yet: Leshner AI. Molecular mechanisms of cocaine addiction. N Engl J Med In press 1997.

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Manuscripts published in electronic format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) l995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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EDITORIAL

"Therapeutic Options and Potential Vaccine Studies Against COVID-19"

The novel coronavirus-2019 (COVID-19) disease was first reported in late 2019 and then turned into a pandemic in the following days and months of 2020. Although the current treatment of COVID-19 is based on anti-inflammatory and anti-viral agents no complete cure is available for this disease. The solution to the pandemic problem seems possible only via two ways, either effective drug therapy or the development of an effective vaccine.

This special issue is gathered very valuable research results or review articles discussing the experiences of clinicians regarding the treatment of COVID-19 or the recent developments in vaccine production. Our first article in this special issue covers the experiences of Turkish clinicians in the treatment of COVID-19 using hydroxychloroquine (HCQ), azithromycin, and favipiravir. Sarikaya et al. have reported that hypertension and diabetes mellitus (DM) have been the most common comorbid reasons. They also have concluded that in patients receiving treatment based on Favipiravir; advanced age, DM, chronic heart disease (CHD), Chronic kidney disease (CKD), troponin-I elevation, and secondary bacterial infections are associated with mortality. Chronic renal failure (CRF) and troponin-I elevation have been defined as predictors of mortality.

In the second article, Mayda et al. have been extensively reported the history of pandemics, opening a new window for better understanding the current situation of human beings in fighting with microbial diseases. The interesting impression of this article on readers is that protection hasn't changed much from İbn-i Sina's "El Kanun fi't Tıb" book to today. These recommendations consist of personal hygiene, distance, and stress-free life.

The third article by Gepdiremen et al. tackles the future therapy of COVID-19 using Viral S-glycoprotein, transmembrane protease serine 2 (TMPRSS2), and angiotensin-converting enzyme-2 (ACE-2) inhibitors as extracellular media components. The authors emphasize the high ability of SARS-Cov viruses in mutation and defend the idea that instead of developing vaccines for each mutant virus, it could be more effective to synthesize de novo drugs like ACE-2 or TMPRSS2 blockers to specifically block spike binding sites of the target cells and prevent virus intrusion, especially at the extracellular media, for future pandemics.

In the fourth article Anacak et al. discuss drug repurposing in the treatment of COVID-19. Repurposing is a process that uses drugs that have been previously defined for certain indications in the treatment of a new disease. authors discuss using several techniques including chemical structure-based methods, ligand-based methods, methods based on bioinformatics data such as transcriptomic based drug repositioning, literature mining-based drug repositioning, and target-based approaches. In conclusion, the authors mention that the recent efforts to develop a treatment for COVID-19, are employing drug repositioning rather than drug and target-based strategies. They also mention that some drugs that were investigated using the disease-based approach strategy did not make a significant difference in clinical studies.

Another drug repurposing study is done by Kesmen et al. which approaches the therapeutic effects of ivermectin an FDA-approved drug as a broad-spectrum antiparasitic compound in COVID-19 treatment. They report that the molecular mechanism of this drug could be related to blocking the nuclear entry of importin alpha and beta-1 (IMPα/β1) mediated viral protein, inhibition of TNF-α and interleukin (IL)-1β production and consequently causing an increase in IL-10 production and activation of the NF-κB pathway. The authors also mention that ivermectin and its derivatives are protected by patents for some indications, hence, patent protection research and appropriate modifications could be useful to come over the obstacles on the way of serving health to the public.

The sixth article of the current special issue is a review entitled "An overview of COVID-19 medicines in current guidelines" by Guler and Gokce which covers guidelines for adult and child patient drug treatment and, also, the Potential antiviral agents used in the treatment process of COVID-19. Their article covers approved doses for Hydroxychloroquine, Azithromycin, Favipiravir, Lopinavir, and Ritonavir. Furthermore, the authors discuss the potential of Remdesivir, Nafamostat, Ribavirin, Oseltamivir, Penciclovir/ acyclovir, Ganciclovir, and Nitazoxanide.

Similarly, Arsoy and Ozdemir have prepared a very comprehensive review of "Current therapeutic interventions for COVID-19". The authors mention that although specific drugs began to be developed, the first potential candidate drugs were drugs such as broad-spectrum antibiotics, antiviral agents, anti-parasitic agents, and interferon, which were planned to be used with similar indications before the pandemic. Their article contains three very useful tables regarding supportive treatment options other than specific medications, classification of various drugs tried in COVID-19, and various combination therapies in COVID-19 disease. These tables are followed by the detailed information about Chloroquine and Hydroxychloroquine and their use in pregnancy and lactation, Mefloquine, Remdesivir, Interferons, Lopinavir/Ritonavir, Ribavirin, Favipiravir Corticosteroids, Vitamin C, Teicoplanin, Ivermectin, Tocilizumab, Oseltamivir, Emetine, Intravenous, Immunoglobulin and other Immunomodulator Agents, Umbilical, Cord Mesenchymal Stem Cell (UC-MSC) Transplantation.

In a very modern approach to anti-viral therapies, Arisoy and Comoglu discuss the possibility of using nano-drug delivery systems in the treatment of COVID-19. Nano drug delivery systems with the ability to target special organs, tissue, or protein can provide higher efficacy with the lower

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doses of drugs which facilitates the drug application in toxic doses. Authors emphasize the ability of liposomes the most known nano-drug delivery systems in targeting lungs. They mention that liposomes are widely used drug carriers by inhalation due to their safety and ability to provide controlled drug release in the lung. These carriers can hold a wide variety of therapeutic molecules for delivery to peripheral airways in large volumes using medical nebulizers. Pressurized metered inhalers, soft spray inhalers, and dry powder inhalers can deliver relatively small amounts of medication to the lungs compared to medical nebulizers that can deliver large volumes using simple liposome preparation techniques. They conclude that it is important for liposomes to be used in COVID-19 treatment due to their biocompatibility, targeting the region, and their ability to create a trapping structure.

In a very important article of our special issue, Prof. Kocyigit discusses the advantages and disadvantages of using Vitamin C in the treatment of COVID-19. The author mentions that normally, Vitamin C is a powerful antioxidant vitamin, however, besides that, it shows a pro-oxidant effect in the presence of pharmacologically high doses of transition metals such as iron and copper. Thus, in antiviral treatment, while it was desired to benefit from both antioxidant and pro-oxidant effects of high-dose Vitamin C it is possible to cause serious damage in on tissue due to the cumulative effect of increased oxidative stress caused by disease inflammation increased the pro-oxidant effect of Vitamın C.

Topcu et al. have presented a very inclusive review on the effects of alkaloids, the natural nitrogen-bearing compounds of plants, fungi and marine organisms in COVID-19 treatment. The authors gather the information regarding the anti-corona virus efficacy of indigo, sinigrin and β-sitosterol, natural alkaloids from Isatis indigotica, showing their activity via inhibiting the cleavage activity of the 3-chymotrypsin-like protease in cell-free and cell-based assay. They also mention the SARS-CoV-2 and SARS-CoV inhibiting activity of two natural alkaloids 10-hydroxyusambarensine and cryptoquindoline from African plants. From Chinese medicinal plant Lycoris radiata, lycorine is being reported in this review as an anti-SARS-CoV alkaloid. In the same manner, antiviral activity of bis-benzylisoquinoline alkaloids; cepharanthine, tetrandrine and fangchinoline in significantly inhibiting human coronavirus HCoVOC43 is being reviewed by the authors. In conclusion the authors point out to the use of two very famous compounds Favipravir and Remdesivir as emergency alkaloids in the fight with viral pandemics.

In the final article of this special issue about treatment strategies, Kaymakci and Guler propose the use of *Cordyceps sinensis* and *Cordyceps militaris* the entomopathogenic fungi which are used for their antiinflammatory, immunomodulatory, lung improving, and antiviral functions in Traditional Chinese Medicine as anti-Corona virus plants. They conclude that according to previous research, *C. sinensis* and *C. militaris* can be effective agents for the prevention and treatment of COVID-19 by immunomodulating, reducing the proinflammatory cytokines, preventing lung fibrosis, improving tolerance to hypoxemia, and inhibiting the viral enzymes.

In the second section of this special issue, potential vaccine studies against COVID-19 are being discussed in two very comprehensive articles by Kazak et.al and Mayda et.al. These authors are agreed in the concept that three pandemics caused by SARS, MERS, and SARS-CoV-2 are believed to have started as a result of bat coronaviruses that crossed the species barrier. Therefore, other outbreaks are likely to occur in the future due to the unlimited supply of coronavirus available in the bat population. Prevention is always the best treatment, and continuous viral surveillance of wild animals is extremely important for potentially emerging CoVs. Clinical trials begin with small safety studies in animals and humans, followed by much larger studies to determine whether a vaccine induces an immune response. As a result of all these, it may take many years for the vaccine to emerge, so fast, reliable and easy-to use viral test kits should be developed on the research side. The continuous development of vaccines and antivirals will provide an assurance of fighting and controlling emerging CoV diseases.

As guest editor, I am so proud and honored of receiving, reading, reviewing, and editing above mentioned scientifically precious works. I need to thank all authors and reviewers who have contributed in the best possible way in generating this special issue. I also owe to thank Prof. Dr. Adem Akcakaya the Editor in Chief of the journal Bezmialem Science. Our success with this special issue would not be possible, had he not laid the groundwork already.

On behalf of all co-editors of Bezmialem Science, I would also like to express my best gratitude to the Rector of the Bezmialem Vakif University, Prof. Dr. Rumeyza Kazancioglu, and the esteemed Board of Trustees for their financial and moral support from this special issue.

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Original Article

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Clinical and Laboratory Evaluation of Patients Diagnosed with COVID-19 Receiving Favipiravir-based Treatment

Favipiravir Temelli Tedavi Alan COVİD-19 Tanılı Hastaların Klinik ve Laboratuvar Değerlendirmesi

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ABSTRACT

Objective: Severe acute respiratory syndrome coronavirus-2 (SARS COV-2); can lead to severe respiratory failure. In this study, factors affecting mortality in Coronavirus disease (COVID-19) patients who were hospitalized with hydroxychloroquine (HCQ), azithromycin and favipiravir treatments were investigated.

Methods: Between March 10 and May 10, 2020, COVID-19 reverse transcription polymerase chain reaction (RT-PCR) positive treatment naive 683 patients were screened retrospectively. Five hundred four patients were followed without treatment or with HCQ treatment. Out of 179 patients, 137 were hospitalized and 42 were directly admitted to the intensive care unit (ICU). Oxygen saturation of these patients is <90%, follow-up (>30/min) and HCQ, azithromycin, favipiravir were started. 35 of 137 patients were transferred to ICU. Mechanical ventilation was provided to 69 (89%) of 77 patients in the ICU. 19 (24%) patients received tosilizumab and 13 (17%) patients received immune plasma. It was divided into two as healing and exitus. Demographic features, comorbid diseases, secondary bacterial infections and acute organ damage were recorded.

Results: Two hundred of the patients were male and their average age was 60.9±16.4 years. HT and diabetes mellitus (DM) were the most common comorbid disease. Acute liver injury was most common. 54 patients became exitus. Exitus group has higher mean age, chronic heart disease (CHD), DM, CRF, acute cardiac damage and secondary bacterial infection are more statistically significant (p<0.05). Exitus status DM 2.17, COPD 2.18, asthma 3.01,

ÖZ

Amaç: Şiddetli akut solunum yetmezliği sendromu-2 (SARS-CoV-2); hafif enfeksiyonlardan ağır solunum yetmezliğine kadar çeşitli tablolara yol açabilir. Bu çalışmada hastaneye yatırılarak hidroksiklorokin (HCQ), azitromisin ve favipiravir üçlü kombine ilaç tedavileri verilmiş COVİD-19 hastalarında mortaliteye etki eden faktörler incelenmiştir.

Yöntemler: 10 Mart-10 Mayıs 2020 tarihlerinde COVİD-19 ters transkripsiyon polimeraz zincir reaksiyonu pozitif tedavi naif 683 hasta retrospektif tarandı. 504 hasta hafif semptomu olup ilaç tedavisi verilmeksizin veya tek başına HCQ tedavisi ile takip edilmiş ve şifa ile sonuçlanmıştı. 179 hastadan 137'si kliniğe, 42'si direk yoğun bakım üntesine (YBÜ) yatırılmıştı. Bu hastaların hepsinin oda havasında oksijen saturasyonu <%90, takipnesi (>30/dk) olup eş zamanlı olarak üçlü kombine ilaç tedavisi (HCQ, azitromisin, favipiravir) başlanmıştı. Klinik takipte 137 hastadan 35'i YBÜ'ye nakledilmişti. YBÜ'deki 77 hastanın 69'una (%89) mekanik ventilatör desteği verilmişti. On dokuz (%24) hastaya tosilizumab, 13 (%17) hastaya ise konvelesan immün plazma uygulanmıştı. Hastalar şifa ile taburcu ve eksitus şeklinde ikiye ayrıldı. Demografik özellikler, komorbid hastalıklar, sekonder bakteriyel enfeksiyonlar ve akut organ hasarları kayıt edildi.

Bulgular: Hastaların 120'si erkek, ortalama yaşları 60,9±16,4'di. HT ve DM en sık komorbid hastalıktı. En sık akut karaciğer hasarı görüldü. Elli dört hasta eksitus oldu. Eksitus grubunda yaş ortalamasının daha yüksek olması, kronik kalp hastalığı (KKH), DM, kronik böbrek yetmezliği (KBY), akut kardiyak hasar ve

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CHD 2.4, CRF 8.3, malignancy 1.6, acute cardiac damage 12.9, secondary bacterial infection development 3.63 times mortality increase statistically significant (p<0.05).

Conclusion: In patients receiving treatment based on Favipiravir; advanced age, DM, CHD, CKD, troponin-I elevation and secondary bacterial infections are associated with mortality. CRF and troponin-I elevation are predictors of mortality.

Keywords: COVID-19, favipirovir, mortality

sekonder bakteriyel enfeksiyon daha fazla görülmesi istatistiksel anlamlı bulunmuştur (p<0,05). Eksitus durumuna DM 2,17, KOAH 2,18, astım 3,01, KKH 2,4, KBY 8,3, malignite 1,6, akut kardiyak hasar 12,9, sekonder bakteriyel enfeksiyon gelişimi 3,63 kat mortalite artışına neden olup istatistiksel anlamlı (p<0,05) saptanmıştır.

Sonuç: Favipiravir temelli tedavi alan COVİD-19 hastalarında; ileri yaş, DM, KKH, KBY, troponin-1 yüksekliği ve sekonder bakteriyel enfeksiyonlar mortalite ile ilişkili faktörlerdir. KBY ve troponin-1 yüksekliği bu hastaların mortalitesi için prediktördür.

Anahtar Sözcükler: COVİD-19, favipirovir, mortalite

Introduction

Coronaviruses (CoV) are RNA viruses that can cause generally mild infections in humans and mammals (1). Severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1) and Middle East respiratory syndrome coronavirus in this group lead to more severe clinical pictures (2). The World Health Organization (WHO) named the new CoV that emerged in Wuhan, China, as (SARS-CoV)-2, and the disease caused by this virus was named COVID-19 (3). SARS-CoV-2, which is a member of the coronavirus family, may cause mild clinical symptoms or lead to severe respiratory failure and may be mortal.

According to the American Center for Disease Control and Prevention and WHO data, approximately 11.6 million people in the world were diagnosed with COVID-19 and more than 539,900 of them died (4).

Protein synthesis inhibitors, RNA polymerase enzyme inhibitors, viral entry inhibitors, human monoclonal antibody inhibitors, anti-inflammatory and immunomodulatory drugs are used in the treatment of COVID-19. Currently, remdesivir and favipiravir, which are anti-viral agents in phase-3 stage, are used in the treatment of the disease (5,6).

In this study, patients who were hospitalized in a tertiary hospital and received the combined hydroxychloroquine (HCQ), azithromycin and favipiravir therapy were evaluated. It was aimed to determine the factors affecting mortality and comorbid diseases in these patients.

Method

This study was approved by the University Health of Science, Hamidiye Scientific Research Ethics Committee (decision number 20/182 dated 15.05.2020).

Study Population

In this study, 683 patients who applied to Ministry of Health Sultan 2. Abdülhamid Han Training and Research Hospital between March 10, 2020 and May 10, 2020 and who were found to be COVID-19 real time-polymerase chain reaction (RT-PCR) positive in nasopharyngeal/oropharyngeal swab samples were retrospectively screened. None of the 683 patients had previously received treatment for COVID-19. 504 of these patients were patients with mild clinical symptoms or asymptomatic, and were followed up on an outpatient basis with hydroxychloroquine treatment for 5 days or without any treatment in line with the COVID-19 guideline recommendation of the Ministry of Health. No mortality was observed in any of the 504 patients; respiratory deterioration requiring initiation of favipiravir therapy did not develop; and resulted in recovery. Out of 683, 179 patients were hospitalized and followed up. Of the 179 patients hospitalized, 137 were admitted to the COVID-19 clinic, the remaining 42 patients were directly admitted to the ICU at the time of admission to the emergency department, after determining the need for ICU. In 137 patients hospitalized in the clinic, dual drug therapy was started as HCQ 2x400 mg and azithromycin 1x500 mg on the first day, HCQ 2x200 mg and azithromycin 1x250 mg for the next 4 days in line with the COVID-19 guideline recommendation of the Ministry of Health. During the follow-up, 2x600 mg maintenance therapy for 4 days was added to patients whose oxygen saturation at room air was <90% and tachypnea started (>30/min) after a loading dose of 2x1600 mg favipiravir on the first day. In 102 of these patients, oxygen saturation in room air increased above 95% after triple combined drug therapy, their tachypneas improved, did not need intensive care, no mortality occurred and were discharged with full recovery. In 35 patients, despite the initiation of triple combined drug and nasal oxygen treatments, their oxygen saturation remained below 90%, and they were transferred to the ICU due to the need for respiratory support. Fourty two patients were directly admitted to the ICU during their admission to the emergency department despite the oxygen support because their saturation was below 90%, respiration rate >30/min or they were found to be hypotensive. In line with the recommendation of the Ministry of Health COVID-19 guideline for patients directly admitted to the ICU, simultaneous triple combined drug therapy (after loading doses of HCQ 2x400 mg, azithromycin 1x500 mg, favipiravir 2x1600 mg on the first day, HCQ 2x200 mg, azithromycin 1x250 mg, favipiravir 2x600 mg for 4 days) was started. 69 of 77 (89%) patients followed in the ICU were given invasive mechanical ventilator support. In addition to triple combined drug therapies, 19 of 77 (24%) patients followed in the ICU were administered tocilizumab and 13 (17%) had convalescent immune plasma therapies.

179 patients who used favipiravir-based triple combination drug therapy included in the study; They were divided into two groups as patients who were discharged after recovery and those who died. Demographic information of the patients such as age and gender, and comorbid diseases such as type 2 diabetes mellitus (DM), essential hypertension (HT), chronic obstructive pulmonary disease (COPD), asthma, coronary heart disease (CHD), malignancy and chronic renal failure (CRF) were recorded. Secondary bacterial infections (blood culture positivity) developed during the follow-up after hospitalization were also recorded to examine the effect on mortality.

At the first admission to the hospital, alanine amino transferase (ALT), aspartate amino transferase (AST), serum urea, serum creatinine, serum troponin-I and estimated glomerular filtration rate (eGFR) were analyzed. A decrease of <20% from baseline eGFR and/or an increase in basal serum creatinine >0.2 mg/dL was considered significant for renal damage; eGFR <15 mL/min/1.73 m2 was considered significant for chronic renal failure; patients with serum ALT and AST levels >40 IU/L were considered significant for acute liver injury (7,8); patients with serum troponin-I levels >30 ng/L were considered significant for acute cardiac damage (9).

In our laboratory, reference ranges are determined by company recommendations; the normal reference range is indicated as ALT of 5-40 U/L, AST of 5-40 IU/L, troponin-I of 0-30 ng/L, urea of 15-44 mg/dL, and creatinine of 0.6-1.4 mg/dL. The eGFR value is calculated using the CKD-EPI system. Biochemical analyzes such as serum urea, creatinine, AST, ALT and troponin-I were measured at the Ministry of Health, Sultan 2. Abdülhamid Han Training and Research Hospital, Mustafa Gültepe Laboratory using in-house Gülhane Askeri Medical Academy kits applied to Abbott Architect C-16000 model autoanalyzer devices.

COVID-19 RT-PCR tests were analyzed in Sultan 2. Abdülhamid Han Training and Research Hospital authorized PCR laboratory with ROTORGEN brand device of QIAGEN using Bio-Speedy COVID-19 RT-qPCR detection kit recommended by Public Health Agency of Turkey (THSK).

Study Limitations

Since data on comorbid diseases are obtained from the anamnesis given by the person at the time of admission, some patients may have missed comorbid diseases. The reliability of the study could be increased by measuring myoglobin, creatinine kinase MB (CK-MB) level with serum troponin-I, and using methods such as myocardial perfusion scintigraphy and echocardiography in the evaluation of acute cardiac injury. The treatments used for comorbid diseases and whether the related diseases are under control could not be evaluated.

Statistical Analysis

Parametric tests were used without normality test due to the compatibility of the Central Limit Theorem (10). Student's t-test was used to compare the means of two independent groups, and the repeated ANOVA test was used to compare the means of more than two dependent groups. Chi-square test was used to evaluate the relationship between categorical variables. The exposure ratio (odds ratio) of variables thought to be associated with exitus status was given. Statistical significance level of the data was given as $p<0.05$. www.e-picos.com New York software and MedCalc statistical package program were used to analyze the data.

Results

One hundred twenty of the patients (67%) included in the study were male and their mean age was 60.9±16.4 years. It was observed that at least one comorbid disease was detected in 104 patients, the most common comorbid diseases were HT and DM. At the time of admission, at least one acute organ injury was detected in 95 patients, with acute liver injury the most common. Fifty four of the study patients (30.2%) died during the follow-up (Table 1).

When the relation of comorbid diseases, clinical findings and socio-demographic characteristics with mortality is evaluated statistically: the mean age of the patients who died was statistically significantly higher than the patients who were discharged with recovery; CHD, DM, and CRF were higher among comorbid diseases; acute cardiac damage at the time of admission and secondary bacterial infection development after hospitalization were higher ($p<0.05$). No statistically significant difference was found between the two groups of patients for gender ($p>0.05$) (Table 2).

When the factors affecting exitus and discharge with recovery are evaluated; DM (OR: 2.17, Cl: 1.1-4.3, $p<0.05$), COPD (OR: 2.18, Cl: 0.75-6.35, $p<0.05$), asthma (OR: 3.01, Cl: 0.79-11.9, $p<0.05$), CHD (OR: 2.4, Cl: 1.2-4.94, $p<0.05$), CRF (OR: 8.3, Cl: 3.01-22.8, $p<0.05$), malignancy (OR: 1.6, Cl: 0.2-9.4, $p<0.05$), acute cardiac damage (OR: 12.9, Cl: 5.5-30.5, $p<0.05$), secondary bacterial infection development during follow-up (OR: 3.63, Cl: 1.5-8.6, $p<0.05$) were statistically significant and it was found that they increased the mortality risk (Table 3).

Discussion

COVID-19 disease can be mild or cause death by causing severe respiratory failure. Since there is no specific treatment for the disease yet, pre-existing treatment agents are used in this disease. HCQ, azithromycin and favipiravir combined triple therapy is recommended for COVID-19 patients with severe pneumonia and hypoxemia in our country. In our study, factors affecting mortality in patients meeting these criteria were examined.

In a study conducted by Guan et al. (11) with 1590 patients, the average age of the patients was 48.9; the mean age of the patient group with comorbidity was 60.8; 25% of all patients and 51.5% of patients with severe clinical picture had at least one comorbid disease; HT was the most common comorbid diseases with a rate of 16.9%. Goyal et al. (12) stated in their study with 393 patients that the mean age of the patients was 62.2, 35.8% of them were obese, HT was the highest comorbid disease with a rate of 50.1% and 10.2% of them died. In a study

Table 1. Distribution of descriptive features of patients with COVID-19 (n=179)

Descriptive Features		n (%)
	$\bar{x}$ ± SD	Min-max
Age	60.9±16.4	21-96
Median (25%-75%)	62 (50-73)	
		n (%)
Gender	Male	120 (67)
	Female	59 (33)
Mortalite	Discharged	125 (69.8)
	Ex	54 (30.2)
DM	No	127 (70.9)
	Yes	52 (29.1)
HT	No	103 (57.5)
	Yes	76 (42.4)
COPD	No	164 (91.6)
	Yes	15 (8.4)
Asthma	No	170 (95)
	Yes	9 (5)
CHD	No	137 (76.5)
	Yes	42 (23.5)
Malignancy	No	174 (97.2)
	Yes	5 (2.8)
Acute liver damage	No	113 (63,1)
	Yes	66 (36.9)
Acute cardiac damage	No	143 (79.9)
	Yes	36 (20.1)
Acute renal failure	No	153 (85.5)
	Yes	26 (14.5)
Chronic renal failure	No	157 (87.7)
	Yes	22 (12.3)
Secondary bacterial infection	No	154 (86)
	Yes	25 (14)

DM: Type 2 diabetes mellitus, HT: Essential hypertension, COPD: Chronic obstructive pulmonary disease, CHD: Chronic heart disease, SD: Standard deviation, min: Minumum, max: Maximum

conducted by Cao et al. (13) with 198 patients, the average age of all patients was 50.1, and 34% of the patients had at least one comorbid disease. It was observed that the rate of at least one comorbid disease in patients hospitalized in the ICU was 52%. While the most common comorbid disease in patients was HT with a rate of 21%, HT (31%) and CHD (26%) were the most common in patients admitted to the ICU. In a study conducted by Richardson et al. (14) with 5700 patients, the mean age of the patients was 63 years, and the most common accompanying disease was found to be HT (56.6%). In this study, it was stated that 41.7% of the patients were obese and 19% were morbidly obese. In our study, while the mean age of our patients was higher than the studies of Guan et al. (11) and Cao et al. (13), it was lower than that of Goyal et al. (12) and Richardson et al. (14). Similar to the studies of these four researchers, we observed that the most common comorbid disease in our study patients was HT. Goyal et al.(12) and Richardson et al. (14) stated that both high average age of the study patients and higher rates of obesity could explain the more frequent occurrence of HT. Because our study patients are of advanced age and have a severe clinical picture, we concluded that we reached HT rates similar to the studies conducted by Goyal et al. (12) and Richardson et al. (14) Ignoring the exclusion criteria, we also concluded that if the COVID-19 RT-PCR positive 683 patients were analyzed, our average patient age and HT rate could be lower.

In a study conducted with 198 patients, Cao et al. (13) stated that the distribution of men and women was equal, 90% of the 19 patients admitted to the ICU were men, and accordingly, the male gender had risk factors associated with the severity of the COVID-19 disease. Jian et al. (15), in a study conducted with 43 patients, stated that the rate of getting the disease was equal in women and men, while they observed that the disease was mortal in 70.3% of male patients and 29.7% of female patients. In addition, it has been stated that the number of men who die is 2.4 times more than women, and that COVID-19 disease causes more mortality in male patients regardless of age. Richardson et al. (14) stated that 60.3% of the patients were male, but gender had no effect on mortality in their study. In our study, we concluded that although the male sex ratio was higher in the whole patient population and the exitus group, gender did not make a statistically significant difference on the severity and mortality of the disease.

Guan et al. (11) stated in their study that COPD, DM, HT and malignancy comorbid diseases led to a 2.68, 1.59, 1.58 and 3.5-fold increase in mortality risk, respectively. In our study, COPD increased the risk of mortality by 2.18, DM 2.17, and malignancy 1.6 times. Our findings were similar to those found by Guan et al. (11) for these three comorbid diseases. Even though it was observed in our study that HT caused an increase in mortality risk 1.7 times, we found that it was not statistically significant. We surmised that the use of antihypertensive therapy and controlling arterial blood pressure in patients with HT may have limited the effect of hypertension on mortality. It is clear that studies to be conducted by evaluating antihypertensive treatment options and treatment compliance rates of patients in large patient groups are needed.

Table 2. Statistical relations between socio-demographic characteristics, acute organ damage and secondary bacterial infections during follow-up and discharge with exitus/recovery status (n=179)

		Discharged with recovery (n=125)	Exitus (n=54)	
Descriptive features		**$\bar{x}$ ± SD**	**$\bar{x}$ ± SD**	**p value**
Age		57.1±16.4	69.7±12.4	**<0.0001**
		n (%)	n (%)	
Gender	Male	83 (66.4)	37 (68.5)	
	Female	42 (33.6)	17 (31.5)	0.78
DM	No	95 (76)	32 (59.3)	
	Yes	30 (24)	22 (40.7)	**0.02**
HT	No	77 (61.6)	26 (48.1)	0.09
	Yes	48 (38.4)	28 (51.9)	
COPD	No	177 (93.6)	47 (87)	0.15
	Yes	8 (6.4)	7 (13)	
Asthma	No	121 (96.8)	49 (90.7)	0.09
	Yes	4 (3.2)	5 (9.3)	
Chronic heart disease	No	102 (81.6)	35 (64.8)	**0.01**
	Yes	23(18.4)	19 (35.2)	
Malignancy	No	122 (97.6)	52 (96.3)	0.63
	Yes	3 (2.4)	2 (3.7)	
Acute liver damage	No	80 (64)	33 (61.1)	0.71
	Yes	45 (36)	21 (38.9)	
Acute cardiac damage	No	116 (92.8)	27 (50)	**<0.0001**
	Yes	9 (7.2)	27 (50)	
Acute renal failure	No	105 (84)	48 (88.9)	
	Yes	20 (16)	6 (11.1)	0.39
Chronic renal failure	No	119 (95.2)	38 (70.4)	**<0.0001**
	Yes	6 (4.8)	16 (29.6)	
Secondary bacterial infection*	No	114 (91.2)	40 (74.1)	**0.002**
	Yes	11 (8.8)	14 (25.9)	

Student's t/Chi-square
*Defines bacteremia that develops in follow-up after hospitalization.
DM: Type 2 diabetes mellitus, HT: Essential hypertension, COPD: Chronic obstructive pulmonary disease, CHD: Chronic heart disease, SD: Standard deviation, min: Minumum, max: Maximum

Rowson et al. (16) reported that 8% of 806 COVID-19 patients had bacterial/fungal co-infections during admission to the hospital. Goyal et al. (12) stated that 130 of 393 patients followed up in ICU received invasive mechanical ventilation support, 5.6% of patients developed bacteremia during follow-up and 10.2% of the patients resulted in death. No data are given on agents that cause bacteremia. 43% of our study patients were followed up in the ICU and bacteremia developed in 14% of these patients during the follow-up, and Klebsiella pneumoniae and Acinetobacter baumanii were the most common bacteremia agents. The patients who developed bacteremia were: those who were followed for more than 7 days under invasive mechanical ventilation support; those with cytokine release syndrome, those with multiple organ failure and shock; and those with underlying comorbid diseases. It was thought that the reason for our secondary bacterial infection rate to be higher than other studies

Table 3. Statistics of factors affecting the exitus state (n=179)

Variable	Odds ratio	Lower (95% CI)	Upper (95% CI)	p value
DM	2.17	1.1	4.3	**Significant (p<0.05)**
HT	1.7	0.91	3.29	Insignificant (p>0.05)
COPD	2.18	0.75	6.35	**Significant (p<0.05)**
Asthma	3.01	0.79	11.9	**Significant (p<0.05)**
CHD	2.4	1.2	4.94	**Significant (p<0.05)**
Malignancy	1.6	0.2	9.4	**Significant (p<0.05)**
Acute liver damage	1.13	0.59	2.18	**Insignificant (p>0.05)**
Acute cardiac damage	12.9	5.5	30.5	**Significant (p<0.05)**
Acute renal failure	0.65	0.25	1.74	Insignificant (p>0.05)
CRF	8.3	3.01	22.8	**Significant (p<0.05)**
Secondary bacterial infection**	3.63	1.5	8.6	**Significant (p<0.05)**

* Significant at p<0.05 level (Odds ratio).
**Defines bacteremia that develops in follow-up after hospitalization.
DM: Type 2 diabetes mellitus, HT: Essential hypertension, COPD: Chronic obstructive pulmonary disease, CHD: Chronic heart disease, CI: Confidence interval, CRF: Chronic renal failure

was that our patients were hypoxaemic, severe pneumonia cases and could be associated with prolonged hospitalization.

In the study by Cao et al. (13), while it was reported that 17% of all patients had acute liver damage, 5% had acute renal failure, 11% had acute cardiac toxicity, 42% of the patients followed in the ICU have acute liver damage, 15% have acute renal failure, and 47% have acute cardiac toxicity. In our study patients, we found lower rates of acute liver injury and acute cardiac toxicity, and similar rates of acute renal failure. In our study, it was determined that 50% of the patients who died had acute cardiac damage and its relation with mortality was statistically significant.

Du et al. (17) reported that 21 of 179 patients diagnosed with COVID-19 who were hospitalized were lost; they stated that being over 65 years old, having a history of cardiovascular and cerebrovascular disease, having CD3 + CD8 + T lymphocyte count ≤75 cells/μL and serum troponin-I level of ≥0.05 ng/mL were associated with mortality. They stated that low CD3 + CD8 + T lymphocyte count and high troponin-I level were predictors for mortality of COVID-19 pneumonia patients. Our mortality rates were higher in our study compared to other studies. We think that our mortality rate was higher due to the fact that our study patients showed severe pneumonia symptoms and were followed for a long time in the ICU with invasive mechanical ventilation support. In our study, serum troponin-I elevation was found 12.9 times higher in the patient group who died, and it was evaluated as a predictor for mortality in COVID-19 pneumonia patients.

Although the number of our patients who received tocilizumab and convalescent immune plasma treatment was small, when the effects of these treatments on mortality were evaluated, 6 of 19 (31.5%) patients who received tocilizumab treatment and 7 of 13 (53.8%) patients who received convalescent immune plasma died. While the mortality rate in the group receiving tocilizumab treatment was similar to our general patient population, the mortality rate was higher in patients who received convalescent immune plasma therapy. When 13 patients who were treated with convalescent immune plasma were retrospectively analyzed, it was observed that the positivity continued for a longer time in recurrent nasopharyngeal/oropharyngeal swab COVID-19 PCR samples. Although it was concluded that long-term nasopharyngeal/oropharyngeal virus load might increase the mortality of patients, it was thought that studies with more patients are needed.

Conclusion

In COVID-19 patients with pneumonia and hypoxemia, where favipiravir-based drug treatment options are used effectively in accordance with current guideline recommendations; advanced age, DM, CHD, CRF, increased serum troponin-I at the time of first admission and secondary bacterial infection development at follow-up were determined as mortality-related factors. CRF and acute cardiac injury have been evaluated to be predictors of mortality in patients with severe pneumonia diagnosed with COVID-19.

Ethics

Ethics Committee Approval: This study was approved by the University Health of Science, Hamidiye Scientific Research Ethics Committee (decision number 20/182 dated 15.05.2020).

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: B.S., Concept: B.S., S.Ç.E., Design: B.S., S.Ç.E., Data Collection or Processing: B.S., Analysis or Interpretation: B.S., S.Ç.E., Literature Search: B.S., S.Ç.E., Writing: B.S.

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References

1. Richman DD, Whitley RJ, Hayden FG. Clinical virology. 4th ed. Washington: ASM Press; 2016:273-91.

2. TG Ksiazek, D Erdman, CS Goldsmith, Zaki RS, Teresa P, Emery S, et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 2003;348:1953-66.
3. WHO. Novel coronavirus - China. Jan 12, 2020. Last Accessed Date: 19.01.2020. Avaialeble from: http://www.who.int/csr/don/12-january-2020-novel-coronavirus-china/en/
4. WHO. Coronavirus disease (COVID-19) pandemic. Last Accessed Date: 07.07.2020. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019.
5. McCreary EK, Pouge JM. Coronavirus disease 2019 treatment: a review of early and emerging options. Open Forum Infect Dis 2020;7:ofaa105.
6. Simsek Yavuz S, Unal S. Antiviral treatment of COVID-19. Turk J Med Sci. 2020;50:611-9.
7. Wang X, Fang X, Cai Z, Wu X, Gao X, Min J, et al. Comorbid chronic diseases and acute organ injuries are strongly correlated with disease severity and mortality among COVID-19 patients: a systemic review and meta-analysis. Research Wash DC 2020:2402961
8. Chai XQ, Hu LF, Zhang Y, Han W, Lu Z, Ke A, et al. Specific ACE2 Expression in Cholangiocytes May Cause Liver Damage After 2019-nCoV Infection. Preprint. Posted online February 03, 2020. bioRxiv 931766. doi: 10.1101/2020.02.03.931766
9. Hui H, Zhang Y, Yang X, Wang X, He B, Li L, et al. Clinical and radiographic features of cardiac injury in patients with 2019 novel coronavirus pneumonia, medRxiv preprint (2020), 10.1101/2020.02.24.20027052
10. Norman G. Likert scales, levels of measurement and the "laws" of statistics. Adv in Health Sci Educ Theory Pract 2010;15:625-32.
11. Guan WJ, Liang WH, Zhao Y, Liang HR, Chen ZS, Li YM, et al. Comorbidity and its impact on 1590 patients with Covid-19 in China: a nationwide analysis [published online ahead of print March 26, 2020]. Eur Respir J 2020;55:2000547 https://doi.org/10.1183/13993003.00547-2020.
12. Goyal P, Choi JJ, Pinheiro LC, Schenck EJ, Chen R, Jabri A, et al. Clinical characteristics of Covid-19 in New York City. N Engl J Med 2020;382:2372-4. Published online April 17, 2020. doi:10.1056/NEJMc2010419
13. Cao M, Zhang D, Wang Y, Lu Y, Zhu X, Li Y, et al. Clinical features of patients infected with the 2019 novel coronavirus (COVID-19) in Shanghai, China. medRxiv. Epub ahead of print 4 March 2020. DOI: 10.1101/2020.03.04.20030395
14. Richardson S, Hirsch JS, Narasimhan M, Crawford JM, McGinn T, Davidson KW, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. JAMA 2020;323:2052-9. Published online April 22, 2020. doi:10.1001/jama.2020.6775
15. Jin J, Bai P, He W, Wu F, Liu WF, Han DM, et al. Yang Gender differences in patients with COVID-19: focus on severity and mortality Front Public Health 2020;8:152.
16. Rawson TM, Moore LSP, Zhu N, Ranganathan N, Skolimowska K, Gilchrist M, et al. Bacterial and fungal co-infection in individuals with coronavirus: A rapid review to support COVID-19 antimicrobial prescribing. Clin Infect Dis 2020;2:ciaa530. doi: 10.1093/cid/ciaa530. Online ahead of print.
17. Du RH, Liang LR, Yang CQ, Wang W, Cao TZ, Li M, et al. Predictors of mortality for patients with COVID-19 pneumonia caused by SARS-CoV-2: a prospective cohort study Eur Respir J 2020;55:2000524.

Review

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History of Epidemics and COVID-19

Salgınlar Tarihçesi ve COVİD-19

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ABSTRACT

In archaeological excavations, it has been reported that formations resembling bacterial fossils are found among the rock layers and they belong to millions of years ago. There are those who have created the flora and preserved us by creating our flora, and those that have brought us to the end, as well as those that help us to form the microorganisms that we share with the same planet, whose existence dates back to ancient times. Although our immune system and medical facilities are trump cards against microorganisms living in the world for much longer than human beings, these are not always enough to protect us, and microorganisms are causing outbreaks that will make history. In this review, we aimed to examine the epidemic diseases experienced by human beings from past to present and their effects.

Keywords: Epidemic, pandemic, COVID-19

ÖZ

Yapılan arkeolojik kazılarda, kaya tabakaları arasında bakteri fosillerine benzeyen oluşumlara rastlandığı ve bunların milyonlarca yıl öncesine ait olduğu bildirilmiştir. Varlıkları bu kadar eski zamanlara dayanan aynı gezegeni paylaştığımız mikroorganizmaların aralarında yediklerimizin oluşmasını sağlayanlar ve floramızı oluşturarak bizi koruyanlar olduğu gibi sonumuzu getirebilecek olanlar da var. İnsanoğlundan çok daha uzun süredir dünyada yaşayan mikroorganizmalara karşı her ne kadar bağışıklık sistemimiz ve tıbbi imkanlar, elimizdeki kozlar olsa da bunlar bizi korumaya her zaman yetmiyor ve mikroorganizmalar tarihe damga vuracak salgınlara neden olarak karşımıza çıkıyor. Bu derlemede geçmişten günümüze insanoğlunun yaşadığı salgın hastalıkları ve etkilerini irdelemeyi amaçladık.

Anahtar Sözcükler: Salgın, pandemi, COVİD-19

Introduction

Epidemics, defined as an increase in the number of individuals infected with an infectious disease in a certain population and in a certain time period, have always been a problem due to microorganisms for a long time (1). Sometimes these epidemics caused many deaths that would cause a high number of deaths, sometimes famines that would lead to other diseases, and sometimes political events that could even stop wars (2). Throughout the history, these microorganism-induced epidemics are mostly caused by bacteria and viruses, as well as also people and, also animals, carry and spread them (3). When we look at diseases transmitted by animals, it was possible that hunter-gatherer people started agriculture and settled down and at the same time, microorganisms in these animals could be transmitted to humans from animal products (milk, feces, etc.) by domestication of animals. In addition to the contamination caused because of these products, the mutations in viruses and the resistance developed by bacteria also played a role in the epidemics to create greater problems (4).

Epidemics Effecting Human History from Ancient Times to the Present

In 430 BC, The Athens plague, which was seen during the Peloponnesian War between Athens and Sparta, and lasted for

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five years and caused the death of 100 thousand people, was recorded as the first pandemic in the ancient times in human history. Libya, Ethiopia, Egypt are the countries affected by Athens plague. Fever, thirst, skin rash and lesions were also detected with the disease and various descriptions like typhoid and Rift valley fever have been made. It is considered that the overpopulation caused by the war was effective in the spread of this first pandemic (5).

In the years 165-180 AC, another epidemic disease, although the cause is still unclear, Antoninus plague, experienced in the Roman Empire and considered to be brought by the soldiers returning from eastern expeditions, was accompanied by the symptoms such as fever, sore throat, diarrhea, and rash, even if it reminds diseases such as smallpox and measles. This epidemic is named after the Roman emperor Marcus Aurelius Antoninus died, and is believed as one of the major plague epidemics, killed 2 thousand people a day and lost 30 percent (5 million) of the total population of the empire. This epidemic started a process that continued until the destruction of the empire, as the power loss of the empire, and led to power wars and civil wars within the empire (6,7).

In 250-270 AC, Cyprian Plague of the Cyprian, named by the Bishop of Carthage, caused the loss of 1 million of the world population, which was approximately 200 million at that time. The epidemic first started in Ethiopia, than spread to Rome, Greece and Syria, and lasted about 20 years. Diarrhea, vomiting, throat ulcers, fever, lesions on the hands and feet, were some of the symptoms of the plague and the pandemic continued to be seen repeatedly in the following 3 centuries (8).

The plague of Justinian was thought to be caused by the bacterium Pasteurella pestie (later named Yersinia Pestis), which started in Europe in 541 and spread to Egypt, Palestine, Syria and from there to Anatolia. This pandemic is thought to have entered the city through mice, which were among the supplies brought into the city by military units. It was thought that the people was infected by the plague which is transferred to humans by the biting of a flying insect named "Xenopsylla" living on mice and caused the infected people to die within a few days. This was interpreted as the first emergence of the bubonic plague, nearly a few hundred daily deaths at the beginning, which was sooner reached to thousands of daily deaths. The disease continued its normal course and disappeared by itself, but 25 to 100 million people were lost till that date (26% of the world's population) (9).

The Japanese Smallpox Outbreak, which occurred in Tokyo between 735-737, spread to neighboring countries, causing the death of approximately one million people. Most of those who died in the epidemic that killed a third of the Japanese population were children (10).

The black plague epidemic, caused by the bacterium Yersinia pestis, which led to one of the most devastating epidemics in history, killed between 75 and 200 million people, one third of the world's population, between 1346 and 1353. After the deaths, the Black Plague epidemic, which caused the God and the church to be questioned in the society, was effective enough to cause a reform in religion (11). Climate change and hot and dry winds facilitated the transport of bacteria by fleas. In addition, the bacteria were spreading to the port cities by the fleas living on the mice on the ships. The beginning of the use of masks began with the theory that doctors suppressed the smell with 55 spices and dried flowers due to the miasma theory. Apart from that, the concept of quarantine was first reported in 1,377 with the enactment of a law that ships and commercial caravans, that want to enter into Ragusa (Croatia-Dubrovnik), coming from places where plague was seen, cannot enter after 1 month disinfection in Mrkan island or Cavtat town (12). "Quarantine", which means 40 days in Italian, has a symbolic and religious meaning, as well as due to the infections that will occur after the puerperium. For the first time in the history of the Ottoman Empire, the official quarantine decision was taken in 1838 and the Ministry of Quarantine was established in 1839 to institutionalize this situation. Urla quarantine island was built together with the French in the 18th century as the first quarantine application area (13).

Europeans who came into contact with the natives of the Americas in the 15th century, infected the microorganisms to the people here. Chickenpox, which killed one third of Europe, killed millions of Native Americans whose immune systems did not recognize this factor. By the 19th century, one in two Native Americans died of diseases derived from Europe (14).

In the 16th century, the epidemic disaster caused by the occurrence of several different diseases such as smallpox ("Cocoliztli" in Aztec) measles and diphtheria, which was carried here by the Spanish from Europe in Mexico, is referred to as "cocoliztli outbreaks". Cocoliztli outbreaks occurred 3 times between 1520 and 1576. Today, it is estimated that the outbreaks claimed to be caused by salmonella bacteria killed nearly 25 million people. This epidemic is thought to have spread from Mexico, from Venezuela to Canada. When the World Health Organization (WHO) launched a campaign for the eradication of smallpox in 1967, it was estimated that there were 10-15 million smallpox cases and 2 million deaths in the world that year. The last case was reported in Somalia in 1977. Smallpox was declared to eradicate in 1980 (15-17).

There have been seven major cholera outbreaks in our history, but the deadliest of these was the third cholera epidemic that occurred between 1852-1860. The main cause of cholera was human feces-contaminated drinking water, but it was understood in the third epidemic. After that, the importance of the boiling of drinking water was suggested. India, where the Ganges river, one of the dirtiest rivers in the world, has been the most affected country. The epidemic spread from India to Afghanistan, Russia, from there to Europe and Africa, and finally to America. The seven cholera epidemics, in which millions of people died, were seen as severe diarrhea (18,19).

After the Justinian Plague and the Black Plague, the Third Plague epidemic, which occurred in 1855-1859, whose origin

was the Chinese Yunnan province, caused the death of more than 12 million people all over the world. The epidemic, whose effects have continued for a century, was carried to the American continent by rats from the far east. At that time, scientists not only found the factors of diseases, but also began to be successful in drug treatments (20). As a result of a visit to Australia after Fiji passed to the British Empire, they brought the disease back to their islands and in 1875, 40 thousand people of whom was the 1/3 of the Fiji population, were lost in the Fiji Measles pandemic (21).

During the First World War between 1914-1918, the typhus epidemic caused by lice carrying typhus bacteria also caused 25 million people in Europe and Asia to become ill, and nearly 3 million people died especially in the Soviet Union countries. While Western countries took precautions against lice and protected themselves, it was seen that many people lost their lives because the Eastern countries failed to do so (22,23).

The Russian flu, which started in 1889 and spread rapidly all over the world, killed 1 million people. It spread from Russia to Europe, from there to America, Australia and Africa. The most important factor in the spread of the epidemic was the railways between Europe and Russia (24). By 1918, the Spanish Flu epidemic caused by the H1N1 influenza virus, which would continue for two years, infected 500 million people in the years following the First World War, causing the death of 50 to 100 million people. The data obtained showed that this number is much more than the number of people who died in war. Although many countries were affected, the most casualties were in India with approximately 18 million deaths. Young age groups were the most affected group in this epidemic. This epidemic, which has affected the whole world, brought to mind today's measures with features such as the use of masks, gargling with salt water and drawing attention to vitamin C consumption. In addition, this epidemic created the need for an international fight against diseases affecting the whole world, and in 1919, today's WHO was established in Vienna, Austria (25-28).

Flu caused by influenza virus (H2N2) appears again as the Asian flu in 1957. Nearly 4 million people died in Asian Flu, which was first seen in Singapore and is estimated to be transmitted to humans by mutation in ducks. The epidemic was stopped with the vaccine found, and within the ten-year Asian flu disappeared in 1968. Asian Flu located in history as one of the most important examples demonstrating the importance and the effect of vaccination (26,29). The cause of the Hong Kong Flu, which killed 1 million people in 1968, was identified as the H3N2 type of Influenza A virus. Babies and the elderly were the most affected by this epidemic, which is a contagious respiratory disease (30).

In the middle of the 20th century, human immunodefiency virus (HIV), which attacks the immune system, especially CD4 T-cells, passed from monkeys to humans, was first seen in Congo in 1959; however, the definitive diagnosis was made in the 1980s (31). According to the United Nations Joint Program on HIV/AIDS (UNAIDS) 2019 report; Since the beginning of the HIV epidemic in the world, 74.9 million people have been infected with HIV, and 32 million have died from AIDS-related diseases (32). This epidemic spreading by blood and sexually, which was stigmatized due to the fact that the first cases were homosexual, was brought under control after the discovery of effective antivirals. With strong protection programs and raising public awareness was also effective in bringing it under control. Although it was in the status of fatal diseases when the first epidemic broke out, it is now in the status of chronic diseases that can be controlled with lifelong drug therapy. Although effective vaccine studies continue to date, no effective vaccine has yet been found (33,34).

The H1N1 Swine Flu epidemic, which started to spread in April 2009, was first seen in Mexico and spread to America and later to 212 countries. WHO announced the start of the pandemic on June 11, 2009. Most deaths from this outbreak occurred in Africa and Southeast Asia. WHO announced that approximately 284 thousand people died and ended on 10 August 2010. The effective antivirals and vaccines take place in ending the epidemic quickly (35).

Until the 2000s, coronaviruses (HCoV-229 E, HCoV-O43, HCoV-NL63, HKU1-CoV) were generally known as the agents that cause upper respiratory tract infections in children (36). In the 2000s, it started to be a problem and cause epidemics as zoonoses. The first serious coronavirus outbreak was the SARS-CoV that started in China in 2002, thought to be caused by bats. The one-year epidemic spread to 29 countries, with 8,096 infected people and 774 deaths. The most striking aspect of this epidemic was early warning. WHO became aware of it in March 2003, and the epidemic stopped in July 2003 (37,38). By 2012, another coronavirus outbreak, MERS-CoV, started in Saudi Arabia. The factor, which was found to be transmitted to humans through bats and camels, spread to 27 countries. It was detected in 2,494 patients and 858 of these patients died. Although it is rarely seen today, cases still continue. Although these two coronaviruses have been brought under control, effective antivirals and vaccines have not yet been found (39,40).

By 2019, it is estimated that coronaviruses reappear as a new type of coronavirus in Wuhan, China, in November, when the incubation period was taken into account. After the incubation period, following the first case of atypical pneumonia on 11 December 2019, 3 more cases were admitted to the hospitals on 17 December 2019. On December 31, 2019, 27 cases of atypical pneumonia reported, most of them working at a seafood and livestock market in Wuhan. As a result of examining the samples taken from the patients, on January 7, it was understood that the virus causing the disease was from the beta coronavirus family, and the virus was named as new coronavirus-2019 (nCoV-2019) (41). It has been reported that the nCoV-2019 genome is 70% similar to the SARS-CoV The nCoV has been officially named as SARS-CoV-2, and the name of the disease it causes has been designated COVID-19 (42,43). Cases have been reported in 216 countries around the world. As of June 2020, more than 10 million

cases and over 500 thousand deaths have been reported. In our country, cases are exceeded 200 thousand and the deaths are over 5,000 (44).

Result

We must always be wary of microorganisms with which we share the world, whose history is older than us. When we look at our past, we see that they cause epidemics with many losses. Each epidemic lead human to new searches and prepares the next with the measures taken. New searches involving drug and vaccine studies sometimes result in positive results, such as the elimination of smallpox with vaccination, and sometimes negative results, like HIV, which takes years and still cannot be obtained from vaccination studies. While these searches continue, sometimes the microorganism loses its virulence and ends the epidemic itself, and sometimes they have resistance against the effective treatment developed, or the microorganism mutates. Regardless of the condition, as long as human beings live, it is as clear as the day that they will come face to face with these microorganisms, and the thing to do is to know the principles of protection from infectious diseases. We see that the ways of protection have not changed much from İbn-i Sina's "El Kanun fi't Tıb" book to today. These recommendations consist of personal hygiene, distance and stress-free life.

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References

1. Hacımustafaoğlu M. Enfeksiyon Hastalıklarında Pratiğinde Salgın Tanımlanması. J Pediatr Inf 2018;12:172-3.
2. Gürel Z, Aslan D. Halk sağlığı bakış açısıyla gıda kaynaklı krizler ve önleme yaklaşımları. Turk Hij Den Biyol Derg 2019;76:361-76.
3. Ergönül Ö. Epidemiology is a field that searchs diseases and their ındicators. Eur Arch Med Res. 2016;32:1-7.
4. Kılınç, GM, Omrak A, Özer F, Günther T, Büyükkarakaya AM, Bıçakçı E, et al. The demographic development of the first farmers in Anatolia. Curr Biol 2016;26:2659-66.
5. Littman, RJ. The plague of Athens: epidemiology and paleopathology. Mt Sinai J Med 2009;76:456-67.
6. See Y, Furuse A, Oshitani SH. Origin of the measles virus: divergence from rinderpest virus between the 11th and 12th centuries. Virol J 2010;7:52.
7. Littman RJ, Littman ML. Galen and the Antonine plague. Am J Philol 1974;94:243-55.
8. Daniel R. Bu Kutsal Tohum: Kuzey Afrika Erken Kiliseleri İnanç, Umut ve Sevgi, (Chester, Ilgın Yayınları, 2010: www.opaltrust.org) Kaynak bulunamadı.
9. The Plague of Justinian. History Magazine. 2009;11:9-12. Bir tek wikipedide bu şekliyle geçiyordu
10. Hurd M. History of epidemics and pandemics, Salem Press Encyclopedia of Health, 2020.
11. Black Death's Gene Code Cracked. Wired. Last Accessed Date: 30.06.2020. Available from.: https://web.archive.org/web/20150426160438/http:/archive.wired.com/medtech/health/news/2001/10/47288
12. Mackowiak PA, Sehdev PS. The Origin of Quarantine. Clinical Infectious Diseases 2002;35:1071-2.
13. Anadolu'nun sağlık kapısı: Urla Tahaffuzhanesi. Erişim Tarihi: 30.06.2020. URL: https://www.aa.com.tr/tr/kultur-sanat/anadolunun-saglik-kapisi-urla-tahaffuzhanesi/1783898
14. Dalia V. Koronavirüs: Karantina nedir, hangi hastalıklar için uygulandı? BBC News-Türkçe, BBC Mundo, Erişim Tarihi: 30.06.2020. URL: https://www.bbc.com/turkce/haberler-dunya-51630138
15. Acuna-Soto R, Stahle DW, Therrell MD, Griffin RD, Cleaveland MK. When half of the population died: the epidemic of hemorrhagic fevers of 1576 in Mexico. FEMS Microbiol Lett 2004;240:1-5
16. Marr JS, Kiracofe JB. Was the Huey Cocoliztli a hemorrhagic fever? Med Hist 2000;44:341-62.
17. Brmen JG, Arıta I, Fenner F. Preventing the return of smallpox. N Engl J Med 2002;348:463-6.
18. Dunnigan M. Commentary: John Snow and alum-induced rickets from adulterated London bread: an overlooked contribution to metabolic bone disease. Int J Epidemiol 2003;32:340-1.
19. Waldman R, Claeson M. Cholera. Encyclopedia Britannica. Last Accessed Date: 09.08.2019. Available from: https://www.britannica.com/science/cholera
20. Bramanti B, Stenseth NC, Walløe L, Lei X. Plague: a disease which changed the path of human civilization. Adv Exp Med Biol 2016;918:1-26.
21. Ofcansky TP. British colonial administration in the Fiji Islands during the 1875 measles epidemic. Adler Mus Bull 1984;10:23-7.
22. Bechah Y, Capo C, Mege J-L, Raoult D. Epidemic typhus. Lancet Infect Dis 2008;8:417-26.
23. Raoult D, Woodward T, Dumler JS. The history of epidemic typhus. Infect Dis Clin N Am 2004;18:127-40.
24. Gregg MB, Hinman AR, Craven RB. The Russian flu, its history and implications for this year's influenza season. JAMA. 1978;240:2260-3
25. Brundage JF, Shanks GD. What really happened during the 1918 influenza pandemic? The importance of bacterial secondary infections. J Infect Dis 2007;196:1717-8
26. Yang W, Petkova E, Shaman. The 1918 influenza pandemic in New York City: age-specific timing, mortality, and transmission dynamics. Influenza Other Respir Viruses. 2014;8:177-88.
27. Arnold D. Dearth and the modern empire: the 1918-19 ınfluenza epidemic in India. Trans R Hist Soc 2019;29:181-200.

28. WHO. WHO issues best practices for naming new human infectious diseases. Last Accesed Date: 08.04.2020. Available from: https://www.who.int/news/item/08-05-2015-who-issues-best-practices-for-naming-new-human-infectious-disease. 8 May 2015.
29. Claire J. History lessons: the Asian Flu pandemic. Br J Gen Pract 2009;59:622-3.
30. Charles CW. Origin and progress of the 1968-69 Hong Kong influenza epidemic. Bull World Health Organ 1969;41:343-8.
31. Cohen O, Cicala C, Vaccarezza V, Fauci AS. The immunology of human immunodeficiency virus infection. In: Mandel GL, Douglas RG, Bennett JE (eds). Principles and Practice of Infectious Diseases. 5th edition. Philadelphia: Saunders: 2000; 107:1374-98.
32. T.C. Sağlık Bakanlığı, Halk Sağlığı Genel Müdürlüğü. Last Accessed Date: 30.06.2020 Avaialeble from: https://hsgm.saglik.gov.tr/tr/haberler/1-aralik-dunya-aids-gunu.html
33. Dökmetaş İ, Hamidi AA. HIV epidemiyoloji. Türkiye Klinikleri J Inf Dis-Special Topics 2016;9:6-11.
34. Haroz D, Zinkernagel DW, Kiragu K. Development and Impact of the Global Plan. Acquir Immune Defic Syndr. J Acquir Immune Defic Syndr 2017;75(Suppl 1):S2-6.
35. Chowell G, Viboud C, Wang X, Bertozzi SM, Miller MA. Adaptive vaccination strategies to mitigate pandemic influenza: Mexico as a case study. PLoS One 2009;4:e8164. PMID:19997603.
36. XinyiLY, LingY,James T, Xiang LD. Human coronaviruses: a review of virus–host ınteractions. Diseases 2016;4:26.
37. Peiris JSM, Phil D, Yuen KY, Osterhaus ADME, Stöhr K. The Severe acute respiratory syndrome. N Engl J Med 2003;349:2431-41.
38. Low DE, McGeer A. SARS-One year later. N engl J Med 2003;349:2381-82.
39. T.C. Sağlık Bakanlığı, Halk Sağlığı Genel Müdürlüğü. Last Accessed Date: 30.06.2020 Avaialeble from: https://hsgm.saglik.gov.tr/tr/bulasici-hastaliklar/mers-co-v/mers-cov-liste/mers-co-v.html
40. İnal S. Middle East Respiratory Syndrome-Coronavirus (MERS-CoV) Enfeksiyonu. Okmeydanı Tıp Derg 2016;32(Ek sayı):37-45.
41. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. N Engl J Med 2020;382:1199-207.
42. Bennett J, Dolin R, Blaser MJ. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 8th ed. Philadelphia, PA: Elsevier/Saunders; 2014.
43. T. C. Sağlık Bakanlığı. 2019-nCoV Hastalığı Sağlık Çalışanları Rehberi. 2020. Available from: https://hsgm.saglik.gov.tr/depo/haberler/ncov/2019-nCov_Hastal_Salk_alanlar_Rehberi.pdf
44. Johns Hopkins University&Medicine. COVID-19 Dashboard by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University. Last Accessed Date: 30.06.2020 Avaialable from: https://coronavirus.jhu.edu/map.html

Review

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ACE-2, TMPRSS2 and Beyond; Promising Targets and Tools for COVID-19 Prophylaxis and Treatment

ACE-2, TMPRSS2 ve Ötesi; COVİD-19 Profilaksisi ve Tedavisi için Umut Vaat Eden Hedefler ve Araçlar

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ABSTRACT

Several repurposing drugs and ongoing vaccine researches to combat Coronavirus Disease-19 (COVID-19) are testing clinically, worldwide. COVID-19 caused by severe acute respiratory failure syndrome-CoV-2, uses angiotensin-converting enzyme 2 (ACE-2) as a functional receptor for entry into the cells, followed by its priming by transmembrane protease serine 2 (TMPRSS2). Most of the ACE-2 expressing cells are alveolar type II pneumocytes. Viral S-glycoprotein, TMPRSS2 and ACE-2 inhibition, as extracellular media components, are potential targets of future therapy. ACE-2 and/or TMPRSS2 blockade is thought to be beneficial in the prevention or treating of this infection which will be the most convenient for pharmacoeconomics and effectiveness, regarding similar future pandemics. Despite substrate-based design and synthesis of ACE-2 inhibitor compounds were presented almost two decades ago, data on renin angiotensin system activation or its blockers, especially ACE-2, are limited by now. Priority must be given to design a convenient vaccine soon, but due to the high mutation ability of such viruses mean that new vaccines may need to be developed for each outbreak. So, *de novo* drugs such as ACE-2 or TMPRSS2 blockers need to be developed which can specifically block spike binding sites of the target cells and prevent virus intrusion, especially at the extracellular media, for future pandemics.

Keywords: ACE-2, COVID-19, SARS-CoV-2, RAAS, TMPRSS2, coronavirus

ÖZ

Başka endikasyonlar için ruhsatlandırılmış birçok ilaç ve aşı araştırmaları, Koronavirüs Hastalığı-19 (COVİD-19) ile savaşta tüm dünyada klinik olarak denenmektedir. COVİD-19'a yol açan ağır akut solunum yolu yetersizliği sendromu, transmembranal proteaz serin 2 (TMPRSS2) tarafından hazırlandıktan sonra, hücrelere giriş için fonksiyonel reseptör olarak anjiyotensin dönüştürücü enzim 2'yi (ACE-2) kullanır. En fazla ACE-2 eksprese edilen hücreler; alveoler tip 2 pnömositlerdir. Gelecekteki tedavilerin potansiyel hedefleri, ekstrasellüler ortam bileşenleri olarak; viral S-glikoprotein, TMPRSS2 ve ACE-2 enzimlerinin inhibisyonu olarak ön plana çıkmaktadır. İleride karşılaşılabilecek benzer pandemiler göz önüne alındığında, enfeksiyonların profilaksisi veya tedavisinde farmakoekonomik kriterler ve etkililik açısından en uygun olarak, ACE-2 ve/veya TMPRSS2 blokajının faydalı olabileceği düşünülmektedir. ACE-2 inhibitörlerinin substrat bazlı tasarım ve sentezleri yaklaşık yirmi yıl önce tanımlanmış olmasına rağmen, renin anjiyotensin sistemi aktivasyonu veya inhibisyonunun ve özellikle de ACE-2 ile ilgili şimdiye kadar elde edilen veriler oldukça yetersizdir. Bu tip salgınlara ilişkin öncelik; uygun aşı tasarlanması ve geliştirilmesi olmakla beraber, bu ve benzer virüslerin yüksek mutasyon kapasiteleri sebebiyle, her salgın için yeni aşı geliştirilmesi gerekliliği aşikardır. Gelecekte ortaya çıkması muhtemel pandemiler için, virüsün hedef hücreye girişini sağlayan dikensi çıkıntıların (spike) bağlandığı ACE-2 ve TMPRSS2 enzimlerini özgün şekilde bloke eden ve özellikle ekstrasellüler ortamda etki göstererek, virüsün hücre içerisine girişini önleyebilecek *de novo* ilaçların geliştirilmesi gereklidir.

Anahtar Sözcükler: ACE-2, COVID-19, SARS-CoV-2, RAAS, TMPRSS2, koronavirüs

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Introduction

Coronavirus Disease-19 (COVID-19) pandemic caused by coronavirus SARS-CoV-2 uses the Angiotensin-converting enzyme 2 (ACE-2) as a functional receptor for entry into the cells and transmembrane protease serine 2 (TMPRSS2) for S protein priming (1). The receptor binding domain of severe acute respiratory failure syndrome-CoV-2 (SARS-CoV-2) has a higher affinity for ACE-2, compared with SARS-CoV with the receptor binding domains of these two coronaviruses share 72% amino acid sequence identity (2). It was demonstrated that 83% of ACE-2 expressing cells were alveolar epithelial type II and those types of cells serve as a reservoir for viral invasion (3). A minority of ACE-2 expressing cells are distributed in the intestine, endothelium, kidney, heart, etc. It is still a mystery that multi-organ invasion of SARS-CoV-2 infection unless viremia could be attributed to it, or not?

This study aims to provide a comprehensive view on COVID-19 and the future expectations to design effective therapeutics and control virus progression. Since effective prophylactics or therapeutics are not available, the development of vaccines and drugs for treatment and prevention of COVID-19 is the matter of emergency. Despite the priority given to the development of SARS-CoV-2 vaccine, new vaccines will need to be developed due to the high mutation rates of this virus (4). Yet, no SARS-CoV-2 therapeutics are currently available, albeit some treatment options which await validation. This could be counted as various antiviral drugs such as remdesivir/favipiravir, immunomodulatory drugs of interleukin-6 (IL-6) and IL-1 antagonists, thalidomide and pirfenidone which may suppress protective acute inflammatory responses, antimalarial drugs of chloroquine, hydroxychloroquine and mefloquine, N-acetyl cysteine which is used to treat human immunodeficiency virus (HIV) and chronic obstructive pulmonary disease, sphingosine 1 phosphate receptor modulator which is approved for the treatment of multiple sclerosis, serine protease inhibitor of camostate mesylate, a low dose of corticosteroids and even some traditional compounds such as cepharanthine.

ACE and ACE-2, serve contrast physiological functions. ACE catalyzes angiotensin I to generate angiotensin II, which binds to and activates angiotensin II receptor type I (AT1R) and constricts blood vessels. ACE-2 inactivates angiotensin II while generating angiotensin 1-7, a heptapeptide having a potent vasodilator function via activation of its MAS receptors (5), as well as the production of nitric oxide and vasodilator prostaglandins (Figure 1). The S-glycoprotein on the surface of coronavirus binds to ACE-2. This leads to a conformational change in the S-glycoprotein and allows proteolytic digestion by host cell proteases (TMPRSS2) ultimately leading to internalization of the virion. Viral S-glycoprotein, TMPRSS2 and ACE-2 inhibition are potential targets of therapy and possible vaccine development (1).

As far as COVID-19 infection is concerned, the data on renin angiotensin system activation or its blockers on the present infection are limited at present. As ACE-2 is the binding site for SARS-CoV-2, its blockade is thought to be beneficial in preventing or treating this infection (6). A retrospective analysis showed reduced rates of death and endotracheal intubation in patients with viral pneumonia who were continued on administration of ACE inhibitors (7). On the other hand, AT1R inhibitors [Angiotensin II Receptor Blockers (ARB's)] and angiotensin converting enzyme inhibitors (ACEi's) are warned of possibly harmful effect due to the increased viral entry into cells en since increased ACE-2 activity might increase viral entry into cells. Also, it was speculated that increased ACE-2 activity could induce conversion of angiotensin II to angiotensin-(1-7), a peptide with potentially protective anti-inflammatory properties (8). It is unclear whether an increasing anti-inflammatory activity is harmful or not in COVID-19 (9). Both ACEi and ARBs were reported to substantially increase ACE-2 activity in cardiac myocytes over one to two weeks in Lewis Rats (10). Olmesartan, an AT1R blocker, when chronically intake for more than one year, found to increase urinary ACE-2 level, which could potentially offer additional renoprotective effects in human (11). Other AT1R antagonist of losartan and olmesartan were shown to increase cardiac ACE-2 expression about three-fold following chronic treatment (28 days) after myocardial infarction induced by coronary artery ligation of rats (12). Conversely, mice with coronavirus induced lung injury showed improvement when treated with an angiotensin receptor blocker, losartan (13). In another study, losartan was shown to upregulate renal ACE-2 expression in chronically treated rats, for more than one year (14). According to the data, mostly obtained from animal models as well as a limited number of human retrospective studies speculate that the treatment with ACE inhibitors and ARB's could cause upregulation of ACE-2 (15). Moreover, Ibuprofen (A non steroidal anti inflammatory drug) and thiazolidinediones (an oral antidiabetic drug group) have also been claimed to do the same (16). Increased expression of ACE-2 speculated to increase the risk of infection with SARS CoV-2, caused serious hesitations in people with hypertension or diabetes who are at already elevated risk of infections. However, there is not enough evidence to support this yet. According to timelines of a basic biological process which were compared in human versus rats and they were found as; 2.5-84 fold faster in rats. Moreover, in the aged phase, one human year was reported to equal 17.1 rat days (17). Thus, it may speculate the upregulation of tissue ACE-2 levels due to the intake of ACEi or ARB's requisite to take a much longer period for human, in respect to laboratory animals. On the other hand, if those drugs cause such fold ACE-2 expression following chronic treatment, it was expected to the increased conversion of angiotensin II to angiotensin-(1-7), stronger vasodilatation and some other protective effects due to the extensive MAS receptors stimulation, but not. Conversely, the doses of ACE inhibitors or ARB's need to be increased in years to reach the same clinical response, in hypertension patients. Moreover, in a retrospective analysis of 112 COVID-19 hospitalized patients with cardiovascular disease in Wuhan-China, no significant difference was found in the proportion of ACEi/ARB medication between non-survivors and survivors (18). So, ACEi/ARB use probably does not affect the morbidity

and mortality of COVID-19 combined with cardiovascular diseases, though more comprehensive retrospective data are needed. Experimental results obtained from laboratory animals cannot be regarded as a criterion for human.

Beyond the testing repurposing drugs on COVID-19 prophylaxis and treatment which are listed above, spike-protein based vaccines, TMPRSS2 inhibition by the administration of the compounds such as nafamostat mesylate and bromhexine hydrochloride, delivering the soluble form of recombinant human ACE-2 which could competitively bind and neutralize the virus, as well as may rescue membranal ACE-2 enzyme, should carry on priority (Figure 2). For SARS-CoV, IC_{50} value for camostate mesylate was found as; 0.68 nM, while for SARS-CoV-2, bromhexine's IC_{50} value was determined as; 0.75 μM and in pulmonary and bronchial epithelial cells, it may reach concentrations of 4 to 6-fold higher than those found in the plasma (19). Also, gabexate and nafamostat mesylate were studied and the latter one reported to inhibit SARS-CoV-2 S-mediated entry into host cells with roughly 15-fold-higher efficiency than camostat mesylate, with an EC_{50} in the low nanomolar range (20). Also, a new generation of TMPRSS2 inhibitor molecules was presented recently with their docking scores (21).

Furin, which is mainly located in lysosomes, is activated by endo-lysosomal acidification and is also taking action in the pathogenesis of some viral infections such as HIV and other coronaviruses, where it cleaves viral enveloping proteins and permeating viral functionality (22). It exerts its action in intracellular, as well as extracellular compartments, which means it exists also as a circulating enzyme (23). Furin inhibitors were reported to block extracellular anchoring activity (cleavage site at the junction between the S1/S2 subunits), but not intracellular processes of viruses (24), which proves the primary tools to combat with SARS-CoV-2 has to be mainly at the extracellular matrix. That's why blockade of another important endo-lysosomal enzyme of cathepsine-L by potent and selective molecules such as; SID 26681509 seems not as important as the other molecules mentioned in that text, as first line drug development priorities (Figure 1).

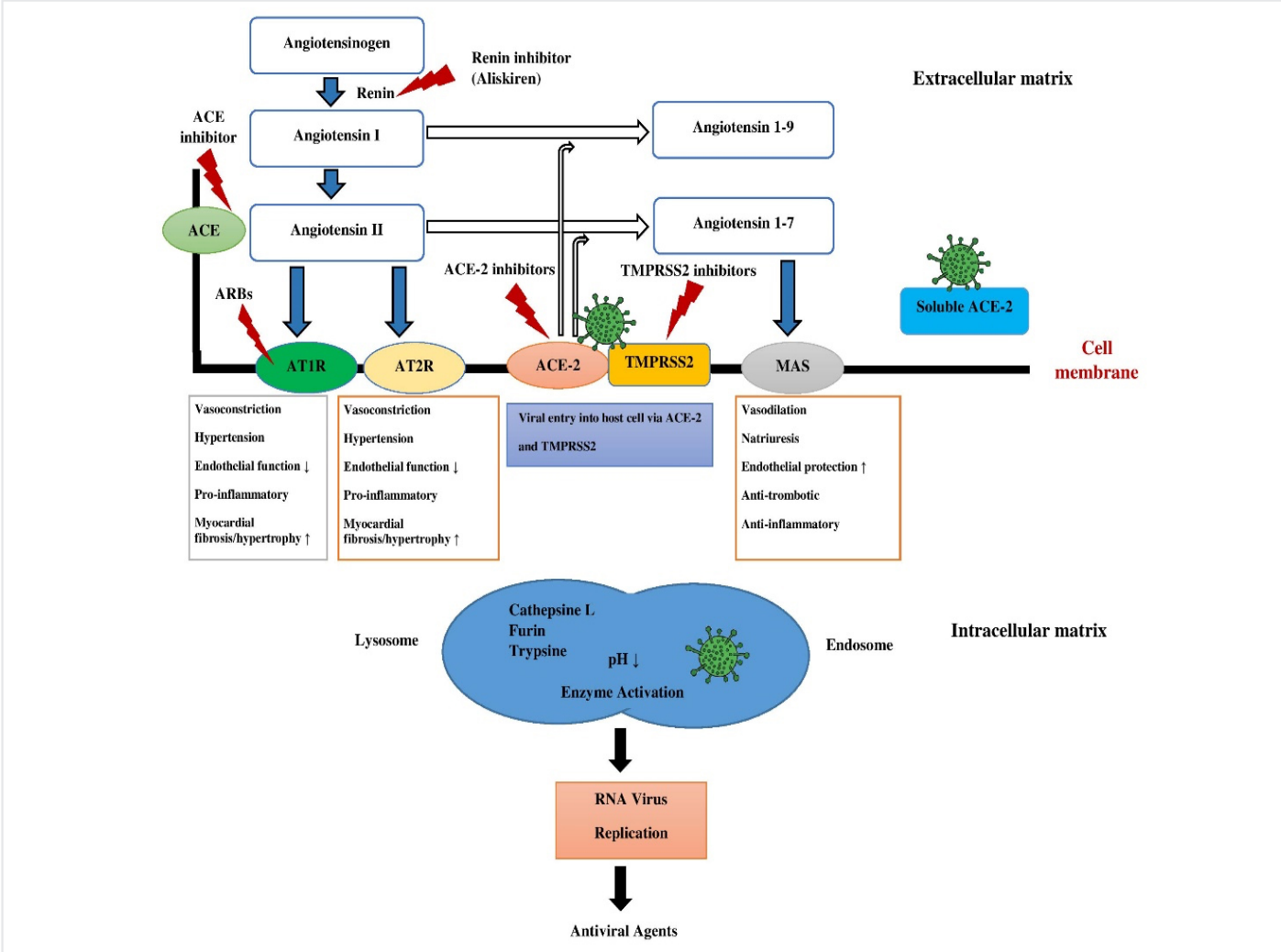

Figure 1. Renin angiotensin system physiology, pharmacology, and the binding domain of COVID-19

ACE: Angiotensin converting enzyme; ACE-2: Angiotensin converting enzyme 2, TMPRSS2: Transmembrane protease serine 2, ARBs: Angiotensin II receptor blockers, AT1R: Angiotensin type 1 receptor, AT2R: Angiotensin type 2 receptor, MASR: Angiotensin (1-7) receptor

Another promising group for *de novo* drug development is ACE-2 enzyme inhibitors, which could be very convenient for cost-effectiveness and potency, regarding similar future outbreaks. Substrate based design and synthesis of ACE-2 inhibitor compounds were firstly introduced in 2002 and that catalytic activity of ACE-2 was found to be not able to suppress by ACE inhibitors because of ACE is a peptidyl dipeptidase while ACE-2 is a carboxypeptidase (25). A few years ago, a new generation of ACE2 inhibitors such as MLN-4760, VE-607, N-2195, DX-600, G-3050, etc. was presented, as still in ongoing preclinical trials (26) (Figure 3). During the acute respiratory distress syndrome, not only ACE/ACE-2 ratio was found to be reduced, but also it could be reported to prevent by angiotensin-(1-7) or angiotensin receptor blockers (27). SARS virus spike protein binding site is located in a different region (suggesting that sequences between amino acids 766 and 771 are required for the insertion of ACE-2 into the plasma membrane) from which is required for the enzymatic function of ACE-2 (28). The successful prevention of the virus cleavage by the new generation of ACE-2 inhibitors is still a matter of doubt. However, due to the importance of ACE-2 in COVID-19, beyond the synthesis of soluble ACE-2, the development of inhibitors mimicking the S-interaction domain in ACE-2 could be employed as a first line therapy for future pandemics. Another major attack of coronavirus which is expected within the next decade, could be more severe than the current one. Therefore, effective therapeutics should be developed based on the already obtained clinical and research information about SARS-CoV-2. The aim of this review was to summarize the possible future preventive and treatment options of coronavirus acquired during this SARS-CoV-2 outbreak.

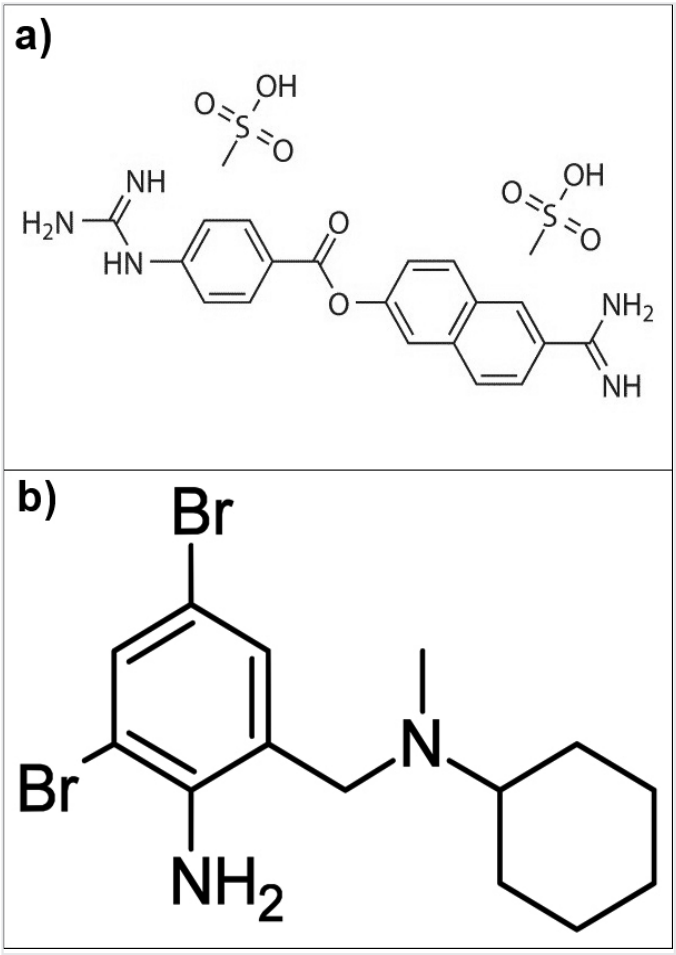

Figure 2. Chemical structure of some transmembrane protease serine 2 (TMPRSS2) inhibitors (a: Nafamostat mesylate, b: Bromhexine hydrochloride)

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Authorship Contributions

Concept: A.G., Design: M.K., Data Collection or Processing: A.G., M.K., Analysis or Interpretation: A.G., Literature Search: A.G., M.K., Writing: A.G., M.K.

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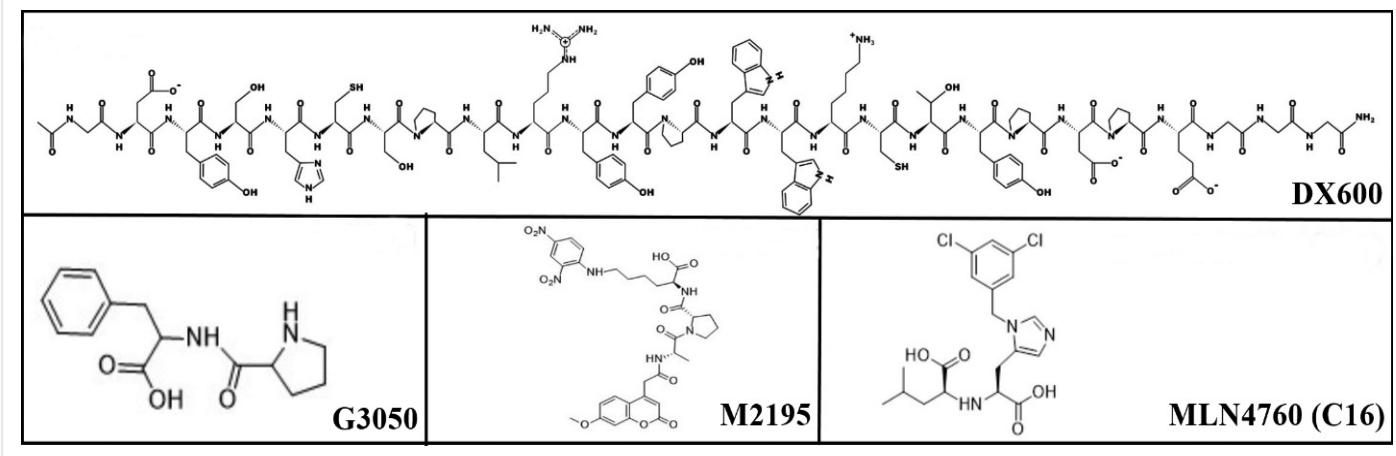

Figure 3. Chemical structure of some angiotensin converting enzyme 2 (ACE-2) inhibitors

References

1. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;S0092-8674:30229-34.
2. Chen Y, Guo Y, Pan Y, Zhao ZJ. Structure analysis of the receptor binding of 2019-nCOV. Biochemical and Biophysical Research Communication. 2020;525:135-40.
3. Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y, Zuo W. Single-cell RNA expression profiling of ACE2, the putative receptor of Wuhan Covid-19. BioRxiv 2020; https://doi.org/10.1101/2020.01.26.919985. [Epub ahead of print].
4. Belongia EA, Skowronski DM, McLean HQ, Chambers C, Sundaram ME, De Serres G. Repeated annual influenza vaccination and vaccine effectiveness: review of evidence. Expert Rev Vaccines 2017;16:1-14.
5. Santos RA, Silva ACS, Maric C, Silva DM, Machado RP, de Buhr I, et al. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. Proc Nati Acad Sci 2003;100:8258-63.
6. Gupta R, Misra A. Contentious issues and evolving concepts in the clinical presentation and management of patients with COVID-19 infectionwith reference to use of therapeutic and other drugs used in Co-morbid diseases (Hypertension, diabetes etc). Diabetes Metab Syndr 2020;14:251-4.
7. Henry C, Zaizafoun M, Stock E, Ghamande S, Arroliga AC, White HD. Impact of angiotensin-converting enzyme inhibitors and statins on viral pneumonia. Baylor University. Medical Center Proceedings 2018;21:419-23.
8. Namsolleck P, Recarti C, Foulquier S, Steckelings UM, Unger T. AT(2) receptor and tissue injury: therapeutic implications. Curr Hypertens Rep 2014;16:416.
9. Aronson JK, Ferner RE. Drugs and the renin-angiotensin system in covid-19. BMJ 2020;369:m1313
10. Ferrario CM, Jessup J, Chappell MC, Averill DB, Brosnihan KB, Tallant EA, et al. Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. Circulation 2005;111:2605-10.
11. Furuhashi M, Moniwa N, Mita T, Fuseya T, Ishimura S, Ohno K, et al. Urinary angiotensin-converting enzyme 2 in hypertensive patients may be increased by olmesartan, an angiotensin II receptor blocker. Am J Hypertens 2015;28:15-21.
12. Ishiyama Y, Gallagher PE, Averill DB, Tallant EA, Brosnihan KB, Ferrario CM. Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. Hypertension 2004;43:970-6.
13. Yang P, Gu H, Zhao Z, Wang W, Cao B, Lai C, et al. Angiotensin-converting enzyme 2 (ACE2) mediates influenza H7N9 virus-induced acute lung injury. Sci Rep 2014;13:7027.
14. Klimas J, Olvedy M, Ochodnicka-Mackovicova K, Kruzliak P, Cacanyiova S, Kristek F, et al. Perinatally administered losartan augments renal ACE2 expression but not cardiac or renal Mas receptor in spontaneously hypertensive rats. J Cell Mol Med 2015;19:1965-74.
15. Li XC, Zhang J, Zhuo JL. The vasoprotective axes of the renin-angiotensin system: Physiological relevance and therapeutic implications in cardiovascular, hypertensive and kidney diseases. Pharmacol Res 2017;125:21-38.
16. Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? Lancet Respir Med 2020;8:e21.
17. Sengupta P. The Laboratory Rat: Relating Its Age With Human's. Int J Prev Med 2013;4:624-30.
18. Peng YD, Meng K, Guan HQ, Leng L, Zhu RR, Wang BY, et al. Clinical characteristics and outcomes of 112 cardiovascular disease patients infected by 2019-nCoV. Zhonghua Xin Xue Guan Bing Za Zhi. 2020;48:E004.
19. Maggio R. Repurposing the mucolytic cough suppressant and TMPRSS2 protease inhibitor bromhexine for the prevention and management of SARS-CoV-2 infection. Pharmacol Res 2020;157:104837.
20. Hoffmann M, Schroeder S, Kleine-Weber H, Müller MA, Drosten C, Pöhlmann S. Nafamostat Mesylate Blocks Activation of SARS-CoV-2: New Treatment Option for COVID-19. Antimicrob Agents Chemother 2020;64:e00754-20.
21. Rahman N, Basharat Z, Yousuf M, Castaldo G, Rastrelli L, Khan H. Virtual Screening of Natural Products against Type II Transmembrane Serine Protease (TMPRSS2), the Priming Agent of Coronavirus 2 (SARS-CoV-2). Molecules 2020;25:E2271.
22. Hallenberger S, Bosch V, Angliker H, Shaw E, Klenk HD, Garten W. Inhibition of furin-mediated cleavage activation of HIV-1 glycoprotein gp160. Nature 1992;360:358-61.
23. Ichiki T, Burnett JC Jr. Post-transcriptional modification of pro-BNP in heart failure: is glycosylation and circulating furin key for cardiovascular homeostasis? Eur Heart J 2014;35:3001-3.
24. Van Lam van T, Ivanova T, Hardes K, Heindl MR, Morty RE, Böttcher-Friebertshäuser E, et al. Design, synthesis, and characterization of macrocyclic inhibitors of the proprotein convertase furin. Chem Med Chem 2019;14:673-85.
25. Dales NA, Gould AE, Brown JA, Calderwood EF, Guan B, Minor CA, et al. Substrate-based design of the first class of angiotensin-converting enzyme-related carboxypeptidase (ACE2) inhibitors. J Am Chem Soc 2002;124:11852-3.
26. Tamargo M, Tamargo J. Future drug discovery in renin-angiotensin-aldosterone system intervention. Expert Opin Drug Discov 2017;12:827-48.
27. Wösten-van Asperen RM, Lutter R, Specht PA, Moll GN, van Woensel JB, van der Loos CM, et al. Acute respiratory distress syndrome leads to reduced ratio of ACE/ACE2 activities and is prevented by angiotensin-(1-7) or an angiotensin II receptor antagonist. J Pathol 2011;225:618-27.
28. Hofmann H, Geier M, Marzi A, Krumbiegel M, Peipp M, Fey GH, et al. Susceptibility to SARS coronavirus S protein-driven infection correlates with expression of angiotensin converting enzyme 2 and infection can be blocked by soluble receptor. Biochem Biophy Res Commun 2004;319:1216-21.

Review

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Drug Repurposing in the Treatment of COVID-19

COVİD-19 Tedavisinde İlaç Yeniden Konumlandırma

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ABSTRACT

The use of drugs that have been previously defined for certain indications in new indications is defined as the repurposing/repositioning of the drug. The requirement of all clinical research steps that starts from healthy volunteers, due to the slowness of new drug discovery, longer time to reach the market, and high cost to develop a new drug, make drug repurposing an attractive pharmacoeconomic solution. Repositioning of drugs becomes even more important, especially in situations where time is vital and emergency in drug development such as pandemics. In this review, we have both summarized the techniques used for drug repositioning and evaluated drugs that have been repositioned in Coronavirus Disease (COVID-19) treatment based on three main strategies, target-disease and drug-based. Considering the availability of the results of pharmacovigilance studies and long-term toxic effects of old drugs provide an important advantage compared to traditional drug discovery in COVID-19 treatment, where sepsis and multi-organ dysfunction can occur.

Keywords: COVID-19, drug repositioning, computer-based approaches

ÖZ

Daha önce belirli endikasyonlar için tanımlanmış olan ilaçların yeni endikasyonlarda kullanımları ilacın yeniden amaçlandırılması/yeniden konumlandırılması olarak tanımlanmaktadır. Yeni ilaç geliştirmek için gereken sağlıklı gönüllülerden başlayan tüm klinik araştırma adımları, bunlara bağlı ilaç keşfinin yavaş ve ilacın piyasaya ulaşma süresinin uzun olması ve dolayısıyla da yüksek maliyet nedeniyle, yeni ilaç keşfi yerine eski ilaçların yeniden konumlandırılması farmakoekonomik bir çözüm olarak ortaya çıkmaktadır. Özellikle zamanın hayati önem taşıdığı ve acil ilaç geliştirilmesi gereken pandemi gibi durumlarda ilaçların yeniden değerlendirilmesi daha da önem kazanmaktadır. Bu derlemede hem ilacın yeniden konumlandırılması için kullanılan teknikleri özetledik, hem de hedef hastalık ve ilaç temelli olmak üzere 3 ana stratejiye bağlı olarak Koronavirüs Hastalığı-19 (COVİD-19) tedavisinde yeniden konumlandırılan ilaçları değerlendirdik. Eski ilaçların farmakovijilans çalışmalarının sonuçları ve uzun vadedeki toksik etkiler dahil görülebilecek yan etkilerin bilinmesi nedeniyle sepsis ve çoklu organ yetmezliğine gidebilen COVİD-19 enfeksiyonunun tedavisinde kullanılacak ilaçların keşfinde ilaçların yeniden konumlandırılması yöntemi geleneksel ilaç keşfine kıyasla önemli bir üstünlük olarak ortaya çıkmaktadır

Anahtar Sözcükler: COVİD-19, ilaç yeniden konumlandırma, bilgisayar temelli yaklaşımlar

Introduction

Pneumonia cases of unknown cause were started to be reported in China on December 31, 2019. A new coronavirus (CoV) severe acute respiratory syndrome CoV-2 (SARS-CoV-2) was identified as the cause of these cases on January 7, 2020. Due to the spread of the virus worldwide, it was declared a global epidemic by the World Health Organization on March 11, 2020. The SARS-CoV-2 virus, which is estimated to be transmitted to humans from the seafood and "wet" animal market in Wuhan city, has stick-like protrusions on its surface. CoV, the enveloped RNA virus, whose name is also given based on these protrusions, are single-stranded

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and positive polarity (1). CoV cause a wide variety of clinical pictures in humans, ranging from the common cold to SARS, and in severe cases, kidney failure or even death. Considering the high mortality rates caused by SARS-CoV-2 and the spread rate of the virus, it is important to develop drugs against this disease quickly.

Development of a drug depends on successful steps following the identification of the target, including literature research for the precursor compound, preclinical studies, phase (I-II-III) studies, approval, and phase IV studies. This process takes a long period of about 15-20 years. Moreover, due to the failures that can be seen in the phase studies of the drug molecule, the efficiency of drug development in the traditional method is low. In order to reduce the cost of synthesis and screening of drug molecules and to shorten the duration, it is necessary to resort to the drug repositioning method, which is defined as the use of available licensed drugs in drug design and development for new therapeutic purposes (2). This method, known as drug repositioning, drug repurposing, drug rescuing, therapeutic switching, drug reprofiling, drug indication expansion, drug indication shift, and drug retasking, is a strategy for defining a new indication to an existing drug or drug molecules under study other than the original medical indication. Acetylsalicylic acid, thalidomide, sildenafil, and minoxidil are the best known examples of repositioned drugs from past to present (3).

Since the efficacy, safety, toxicity, and clinical effect information of the approved drugs are already available, their safety has been tested in early studies, and their pharmacology and formulations are known, the introduction of these drugs in a new indication within the scope of repositioning is rapid. One of its most important advantages is the low failure risk. Also, the ability to reduce the required clinical trial steps reduces the time and cost of the drug to reach the market. Due to these advantages, the drug repositioning strategy is one of the most powerful methods in developing drugs for the treatment of diseases such as COVID-19, which is considered as a global epidemic that causes the death of many people by spreading rapidly and affects the whole world negatively. However, despite all its advantages, drug repositioning has some difficulties. First, the dose required to treat the new disease may differ from the disease in the original indication, and in this case, omission of phase I clinical trials, one of its advantages against *de novo* drug development, is not possible. Second, when new formulations and new distribution mechanisms of existing drugs are in question in new and specific diseases, seldom it is required to perform pharmaceutical and toxicological studies again. Third, patent rights issues can become much more complex due to the lack of legal expertise in drug repositioning (4).

Techniques Used in Drug Repositioning

Chemical Structure-based Methods

Molecular docking is a target-based approach that allows us to model the behavior of two interacting molecules (protein/ligand, protein/protein) at the atomic level and elucidate the basic bonding mechanisms. This approach uses 3D models of the target and candidate ligand. The basic steps of the molecular docking process are: 1- Creating the 3D structure of the target and ligands, 2- Placing the ligand in the target's binding site, and 3- Calculating the binding affinity. The last step is to score how strong the interaction between the target and the ligand is (5). Various computer softwares are available to perform these basic steps.

In molecular docking studies, virtual screening of large structural libraries containing chemical formulas of existing drugs and/or drug candidate compounds is performed, and interactions between a molecular target of interest and candidate compounds are evaluated. Virtual scanning provides time and cost advantages; because it eliminates the need to purchase hundreds of compounds to synthesize new molecules. The combination of virtual scanning and drug repositioning methods gives promising results in drug development studies. For example, by scanning protein databases, the antiparasitic drug mebendazole was also shown to be a vascular endothelial growth factor receptor 2 inhibitor by the molecular coupling method (6). Although molecular docking is a very popular and widely used approach, it also has several limitations. The 3D structure of the target must be present before molecular coupling can be used. In the absence of this data, homology modeling can be performed for the target, but in this case, the obtained structure of the target may not be exactly the same as the reality. In addition, molecular docking studies can become inefficient due to lengthy calculations in large-scale analyzes (7).

Ligand-based Methods

Ligand-based methods include pharmacophore modeling, quantitative structure-activity relationship (QSAR), and reverse docking methods. These approaches are based on the idea that structurally similar compounds can exhibit similar biological properties (7).

The pharmacophore modeling method is applied to analyze the target's binding pocket or compounds known to interact with the target of interest. A list of available compounds is then made, and candidate compounds are compared with a scoring system and the pharmacophore model to find compounds similar to the pharmacophore model (8). Pharmacophore based drug repurposing yields results with higher accuracy than molecular docking and is considered computationally less demanding. However, only a very small fraction of the virtual results obtained as a result of pharmacophore modeling contain real bioactive compounds. Therefore, optimization and validation become even more important.

Quantitative QSAR methods use the relationship and interactions between the target protein and the ligand, and these interactions play key roles in analyzing the properties of drugs. QSAR models are based on the principle that molecules with similar structures show similar biological activity. It also uses statistical methods to associate drug-target interactions with different molecular properties. Sufficient data is needed to allow the extraction of necessary features to apply this approach. The main difference

between the pharmacophore model and QSAR methods is that the pharmacophore model is based only on the basic properties of a ligand, while in QSAR methods, both the essential properties of the ligand and the properties related to the activity between the ligand and the target are taken into account (9).

In the reverse docking method, the library of clinically proven targets within the scope of repositioning drugs is screened for a single ligand in order to expand the indications of the compounds. Based on a specific scoring system, the result of this screening is taken as a list of goals. According to this list, the highest-scoring targets may be used for drug repurposing because the highest scoring targets have a higher potential to bind to the specific ligand (10).

In order to apply the reverse docking method within the scope of drug repurposing, information about the potential ligand-binding sites as well as the structural library of target proteins is required (11). In the absence of this information, various computer programs can be used to predict the ligand-binding sites, but the sensitivity of the results will be questionable (12). The ligand must be an approved drug or an experimental or research drug, and this information must be obtained from databases such as DrugBank, National Institutes of Health (NIH) Chemical Genomics Center Pharmaceutical Collection, and Chemical Database of Bioactive Molecules. Databases such as Potential Drug Target Database are available for reverse docking (13).

Methods Based on Bioinformatics Data

Transcriptomic Based Drug Repositioning

Rapid advances in genomics have allowed the acquisition of large-scale genomic and transcriptomic data both in healthy humans and animal tissue/cell samples and in a variety of diseases or disease models. Based on the transcriptomic data containing the list of genes whose expression level decreased or increased in biological samples obtained from healthy and sick individuals under different experimental conditions, whether drug therapy changes the expression profiles of the evaluated genes could be determined (14). This approach to using transcriptomic data available for drug repurposing has been used successfully in many cases and has given promising results. For example, Okada et al. (15) scanned gene loci that may be associated with rheumatoid arthritis and identified existing drugs associated with genes in this region. This development has revealed the potential of these drugs to be repositioned for rheumatoid arthritis (15).

Genetic Variation Based Drug Repositioning

Genome-wide association studies (GWAS) are a new approach that involves the rapid screening of all DNA sequences of many people to find genetic variations associated with a particular disease. Researchers use this data to identify genes associated with a particular disease trait and to investigate how these variations affect drug responses. GWAS can detect thousands of single nucleotide polymorphisms simultaneously, and this data source is used to make associations about complex diseases such as Alzheimer's disease and multiple sclerosis. GWAS can detect gene-disease relationships that are valuable within the scope of drug repurposing and can bring new indications to existing drugs (16). By combining existing data of 7,000 genes and 2,500 diseases, Nelson et al. (17) revealed 16,000 disease-gene relationships and 19,000 drug targets-disease relationships and showed that selecting genetic targets can double the success rates of clinical drug development programs.

Literature Mining Based Drug Repositioning

Today, information has spread to many different journals, databases, or scientific platforms, and the network is expanding day by day. Literature mining is used to identify and connect many seemingly unrelated indirect links and relationships of available scientific literature. It is possible to discover drugs and identify new drug indications by using all available and accessible literature with the help of computerized literature-based approaches (18). In this method, based on a large scientific literature, the links between relevant data are analyzed to identify and describe the basic molecular mechanisms of a disease.

One of the sensitive points of literature-based drug discovery and repositioning is the selection of appropriate information sources. Information about abstracts, publications, gene and disease interactions, and protein-protein interactions in Pubmed, which is a very rich database in life sciences and biomedical fields, is frequently used in drug repurposing studies.

Drug Repositioning Strategies and COVID Treatment

Drug repositioning using the techniques reported above is possible with three different strategies as target-based, disease-based, or drug-based (19). Drugs that have the potential to be re-evaluated using these approaches in the treatment of COVID-19 are listed below.

Target Based Approach

In the target-based approach relationship between a drug molecule and protein interactions is examined. In this approach, defining the use of a drug in a new indication depends on a well-defined goal. In drug research and development studies for COVID-19 in line with the target-based approach, angiotensin-converting enzyme 2, Spike protein, Transmembrane Serine Protease 2 (TMPRSS2), severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) main protease (Mpro) also known as 3C-like protease (3-chymotrypsin-like protease or 3CLpro), papain-like protease (PLpro), ribonucleic acid (RNA)-bound RNA polymerase (RdRp), E protein, and Helicase were determined as target proteins. The cluster of differentiation 147 (CD147) receptor has also been identified as a therapeutic target (20,21).

Approaches Targeting the ACE2 Enzyme

SARS-CoV-2 was shown to perform membrane fusion by attaching to ACE2 through the receptor binding site of spike proteins and thus can enter the human cell (22). A study used a library of antiviral compounds, including 2,924 molecules in the ZINC database and 78 commonly used antiviral drugs in the market, for target-based analysis. These libraries included

those subjected to clinical trials for SARS-CoV-2, and whether the target was screened among the targets mediating the possible effects of these candidate drugs was investigated. As a result of this screening, 22 molecules used as drugs were also found to have ACE-2 enzyme inhibition potential. These were foscarnet, estradiol benzoate, thalidomide, troglitazone, cefamandol, losartan, benzylpenicilloyl G, ergotamine, methotrexate, cefmenoxim, carboprost, gadobenate dimeglumine, trandolapril, folinic acid, tranilast, meclizin, ziprasidon, dicumarol, riboflavine-5-phosphate, silibinin, carmine, and cinnarizine (23).

Approaches Targeting Spike Protein of the SARS-CoV-2 Virus

REGN-3051 and REGN-3048 are monoclonal antibodies targeting the spike protein of the MERS-CoV virus. Based on the antigenic site similarities between MERS-CoV and SARS-CoV-2, it is thought that these monoclonal antibodies may have the potential to target SARS-CoV-2. The phase 1 clinical study (NCT03301090) with these monoclonal antibodies has been completed.

In the study conducted by Wu et al. (23), 29 molecules targeting the spike protein were found as a result of target-based virtual ligand scanning. These are azlocillin, penicillin, liothyronine, gliclazide, telmisartan, cefsulodin, seliprolol, levodopa, sorafenib, posaconazole, itraconazole, iloprost, etofilin clofibrate, fenofibrate, sildenafile, vardenafil, dabigatran etexilate, pioglitazon, vitamin C and β -carotene and rescinnamine (23).

In another study, 7,922 molecules from the NPC database were screened by targeting the SARS-CoV-2 Spike protein receptor binding site. According to this study, denopamine, bometolol, naminterol, rotigaptid, and benzquercin molecules were determined as potential ACE2 inhibitors (24).

Approaches Targeting TMPRSS2

SARS-CoV-2 uses TMPRSS2 to prepare spike (S) protein (25). Camostat, previously approved for the treatment of chronic pancreatitis and postoperative reflux esophagitis, has been shown to inhibit TMPRSS2 via serine protease inhibition. A study demonstrated that SARS-CoV and human coronavirus (NL63) infections were partially prevented by inhibiting TMPRSS2 in HeLa cells (26). Therefore, the effectiveness of Camostat against the SARS-CoV-2 virus is being investigated. In an in vitro study, Camostat has been shown to significantly reduce the infection of Calu-3 lung cells by SARS-CoV-2 (25).

In the study conducted by Wu et al. (23), 26 drug molecules were found as potential TMPRSS2 inhibitors as a result of target-based virtual ligand scanning. These were abacavir, fulvestrant, pivampicillin, metacillin, montelukast, cefoperazone, ceftazidime, fludarabin, azlocillin, ceftizoxime, ceforanid, ethacrynic acid, cefotaxime, methotrexate, rosoxacin, alitretinoin, azacitidine, telmisartan, olmesartan, levofloxacin, clindamycin, trimethobenzamide, acrivastine, glibenclamide, and tetrahydrofolic acid.

Elmezayen et al. (27) also analyzed 4,500 molecules obtained from ChEMBL, DrugBank, and Selleckchem databases and 30,000 molecules from ZINC15 database by target-based scanning in another study. As a result of this analysis, 4 molecules were determined as potential TMPRSS2 inhibitors. Of these molecules, Rubitecan is a topoisomerase inhibitor, and Loprazolam is an anxiolytic. The other two molecules are ZINC000015988935 and ZINC000103558522 that have the capacity to be drugs (drug-like compounds) in the ZINC15 database (27).

Approaches Targeting the SARS-CoV-2 Master Protease

SARS-CoV-2 Mpro also known as 3C-like protease (3-chymotrypsin-like protease or 3CLpro) is involved in activating viral proteins produced in the host cell. It performs this activation by cutting unfolded viral proteins (28).

In the study conducted by Wu et al. (23), 27 drugs were identified as potential 3CLpro inhibitors as a result of targeted virtual ligand scanning in the ZINC database. These were lymecycline, chlorhexidine, alfuzosin, cilastatin, famotidine, almitrin, progabid, nepafenac, carvedilol, amprenavir, tigecycline, demeclocycline, montelukast, cefpyramide, pheneticillin, candoxatril, nicardipine, estradiol valerate, pioglitazone, conivaptan, telmisartan, doxycycline and oxytetracycline, carminic acid, mimocin, flavine mononucleotide and lutein molecules. (23). Bagherzadeh et al. (29) screened 160 antiviral molecules from the DrugBank database against the SARS-CoV-2 master protease target. As a result of this screening, 21 molecules were found as potential SARS-CoV-2 major protease inhibitors. These were antiviral drug molecules like adafosbuvir, amprenavir, asunaprevir, atazanavir, boseprevir, darunavir, galidesivir, indinavir, ritonavir, sofosbuvir, sorivudine, telaprevir, tenofovir-alafenamide, inarigivir, merimepodib, nelfinavir, remdesivir, taribavirin, and molecules whose phase 2 studies were completed like L-756423 and TAS-106. This study also screened Papaine-Like Protease target. Thus, seven molecules were found that are potential dual inhibitors for both SARS-CoV-2 master protease and papain-like protease. Among them, inarigivir, merimepodib, nelfinavir, remdesivir, taribavirin, and valganciclovir are the molecules used as drugs. TAS-106 molecule, whose phase 2 study has been completed, was also included in the list (29).

Durdagi et al. (24) found eight molecules as potential SARS-CoV-2 main protease inhibitors. These were rotigaptid, telinavir, ritonavir, terlakiren, cefotiam, cefpyramid pimelautide, and pinokalant (24).

In another study, Elmezayen et al. (27) screened based on the SARS-CoV-2 Main Protease target. As a result of this screening, four molecules were found as potential SARS-CoV-2 Main Protease inhibitors. These were penicillin antibiotic talampicillin and antipsychotic lurasidone. The other two molecules in the list were ZINC000000702323 and ZINC000012481889 molecules, which have the capacity to be drugs (27).

Approaches Targeting Papain-like Protease

Papain-like proteinase (PLpro) is responsible for the cleavage of the N-terminal of the Replicase poly-protein. With this cleavage, Nsp1, Nsp2, and Nsp3, which are responsible for regulating

virus replication, are released. It has also been confirmed that PLpro is responsible for antagonizing the innate immunity of the host (30).

As a result of target-based virtual ligand scanning Wu et al. (23) found that 29 drugs could be potent papain-like protease inhibitors. These were ribavirin, valganciclovir, thymidine, oxprenol, doxycycline, acetophenazine, lopromid, riboflavin, reproterol, chloramphenicol, chlorphenecin carbamate, levodropropizine, sefamandol, floxuridine, tigecycline, pemetrexed, ademethyldilazine, masoproxol, isotretinoin, dantrolene, nicardipine, sildenafile sulphasalazine, silibin, glutathione, hesperetin; L (+) - ascorbic acid, aspartame and finally 2.2'-cyclotidine molecules (23).

Bagherzadeh et al. (29) also found 21 potential inhibitor molecules in their screening study for a papain-like protease target. These were drugs such as penciclovir, lopinavir, maribavir, ribavirin, vidarabin and zanamivir, inarigivir, merimepodib, elsulfavirin, faldaprevir, famciclovir, nelfinavir, remdesivir, taribavirin, valganciclovir, ascorbic acid, GS-6620, cytarabine drugs 2-phase deoxyglucose and TAS-106 molecule whose phase-2 study has been completed. In addition, the 5-guanylmethylene bisphosphonate molecule used in experimental studies is also on the list (29).

Approaches Targeting RNA Dependent Rna Polymerase

Nsp12, a protein conserved in the coronavirus, is an (RNA)-dependent RNA polymerase (RdRp) and is the vital enzyme of the coronavirus replication/transcription complex (31).

In the study conducted by Wu et al. (23), 20 molecules were identified as potential RdRp inhibitors among drug molecules as a result of target-based virtual ligand screening. These wee valganciclovir, chlorhexidine, ceftibuten, fenoterol, fludarabine, itraconazole, cefuroxime, atovaquone, chromoline, pancuronium bromide, cortisone, tibolone, novobiosin, idarubicin, bromocriptine, diphenoxylate, benzylpenisylloil G polylysine, dabigatran etexilat, chenodeoxycholic acid, and syllibinin (23).

Approaches Targeting the E Protein

E protein (E-channel) has important biological functions for the structural integrity of coronavirus and host virulence. E (Envelope) protein forms the viral envelope. Wu et al. (23) identified 23 potential inhibitor molecules as a result of virtual ligand screening based on the E protein target. These were ritonavir, amprenavir, atazanavir, valrubicin, montelucast, candesartan, sofalcon, ceftizoxime, xipamide, piperacillin, cefpyramid, demecarium bromide, sulfasalazine, quinapril, chlorhexidine, benzylpenicilloyl G polylysine, ergotamine, flusemethanethane, flushedrine, hyperforin and lutein molecules (23).

Approaches Targeting the Helicase Protein

Helicase (Nsp13) is a multifunctional protein. The SARS-Nsp13 sequence has been reported to be a necessary component for coronavirus replication. Therefore, it has been identified as a target for antiviral drug discovery.

In their target-based virtual ligand screening studies, Wu et al. (23) found that 27 drug molecules could be potential inhibitors of the Helicase enzyme. These were carbenicillin, olsalazine, imipenem, vidarabin monophosphate, gadobenate dimeglumine, benzylpenicillin, etacrinic acid, dienestrol, limexiline, tolmetin, folinic acid, 5-aminolevulinic acid, acetylcysteine, eprosartan, cefsulodin, saquinol, methotrexin, vitrexavine, dabigatran, antrafenine, ceftazidime, canrenoic acid, glutathione , and gadoteridol (23).

Approaches Targeting CD147 Receptor

The spike protein of the SARS-CoV-2 virus binds to the CD147 receptor, a receptor on target cells, thus infecting the target cell. For this reason, the CD147 receptor has been determined as a target in drug research and development studies for SARS-CoV-2 virus. Meplazumab, an anti-CD147 monoclonal antibody, has been shown to be effective in the treatment of COVID-19 (20).

Disease-based Approach

In the disease-based approach, the purpose is to bring new indications to drugs by examining the characteristics and common profiles of different diseases. A drug may be indicated for use in another disease with similar pathology to the pathology of the disease for which that drug is indicated. In this context, the disease-based approach is based on the common molecular pathology similarity between diseases or on related diseases (19).

Antiviral Drugs

Within the scope of the disease-based drug repositioning approach, taking into account that COVID-19 is based on an RNA virus, antiviral drugs have been used. Antiviral therapy is known to be beneficial when initiated earlier in the course of the disease in both influenza and SARS (32). In line with information obtained from SARS and Middle East Respirotary Syndrome (MERS) outbreaks, repositioning existing antiviral drugs to combat the current coronavirus epidemic is considered one of the fastest ways to achieve results. Despite the urgent need to find an effective antiviral treatment for COVID-19 through randomized controlled trials, some agents are used worldwide, based on in vitro or in vivo evidence or observational studies. However, there is a need for precise clinical research data on drugs considered to be used for safe and effective treatment of the disease.

Remdesivir

Remdesivir is a broad-spectrum antiviral agent developed by Gilead Sciences for the treatment of Ebola RNA virus infection in 2017. Animal studies have shown that remdesivir can effectively reduce the viral load in the lung tissue of MERS-CoV infected mice, improve lung function and alleviate pathological damage to lung tissue (33). Wang et al. (22) also found that remdesivir strongly blocks SARS-CoV-2 infection at low micromolar concentrations (34). In the preliminary data of a clinical study conducted with 1063 patients from 10 different countries, remdesivir was found to be superior to placebo in shortening the recovery time and in the treatment of lower respiratory tract infection

in adults hospitalized with COVID-19 (NCT04280705) (35). Many clinical trials for remdesivir are ongoing. FDA, approved (Emergency Use Authorization) remdesivir, whose effectiveness in COVID-19 treatment has not been absolutely demonstrated, only as a research drug, and as an emergency drug for suspected, laboratory-confirmed or severe COVID-19 treatment as Emergency Use Authorization. The European Medicines Agency Human Medicinal Products Committee reviewed data on the use of remdesivir for COVID-19 treatment on May 15, 2020, and put forward recommendations on how to use remdesivir.

Favipiravir

Favipiravir is a new type of RNA-dependent RNA polymerase (RdRp) inhibitor drug, approved in 2014 for use in the treatment of pandemic influenza virus infections in Japan (36). Favipiravir, which is activated after being taken into the cell and is accepted as a substrate by viral RNA polymerases, is thought to inhibit RNA polymerase activity. China approved the effectiveness of favipiravir in the treatment of COVID on March 17, 2020. Phase 3 studies on favipiravir in Japan were initiated on March 31, 2020, and a Phase 2 study in America was initiated on April 9, 2020 (NCT04349241, NCT04402203, NCT04376814, NCT04310228). Favipiravir is approved for use in the treatment of COVID-19.

Lopinavir + Ritonavir Combination

Lopinavir is a highly specific protease inhibitor for HIV-1 and HIV-2; ritonavir increases the plasma concentration of lopinavir through inhibition of cytochrome P450 (37). Ritonavir + lopinavir combination has been shown to be beneficial in patients with SARS infection, and a randomized controlled clinical trial was initiated in 2018 to evaluate the effect in patients with MERS-CoV infection in combination with IFN (MIRACLE trial; NCT02845843) (38). In a randomized, controlled, open-label study, 199 inpatients with severe SARS-CoV-2 infection were randomized to receive lopinavir + ritonavir and standard care for 14 days or only standard care. According to the results of the study, there was no significant difference between combination therapy and standard therapy (39). However, in another study, patients in the group in which interferon was added to lopinavir 400 mg + ritonavir 100 mg every 12 hours for 14 days, and alternately 3 doses x 8 million IU interferon beta-1b or ribavirin 100 mg were shown to be more effective at improving symptoms, reducing viral load and shortening the length of hospital stay than the group receiving the combination of lopinavir 400 mg and ritonavir 100 mg (40).

Umifenovir

Umifenovir is a membrane fusion inhibitor that targets viral entry. Umifenovir has demonstrated antiviral activity in vitro on widely spread virus strains such as Ebola virus, human herpesvirus 8, hepatitis C virus (41). *In vitro* activity of umifenovir against SARS-CoV-1 and SARS-CoV-2 were reported. (42). There are randomized clinical trials in China investigating the efficacy and safety of umifenovir against COVID-19 (NCT04252885, NCT04260594).

Bemcentinib

Bemcentinib is an AXL kinase inhibitor, previously studied in cancer patients and has been shown to be safe and well-tolerated. It has also been reported to exhibit potent antiviral activity in preclinical models against a variety of enveloped viruses, including Ebola and Zika virus. Recent data has expanded this to include SARS-CoV-2. A multi-center Phase II clinical study is being followed in England to examine its effectiveness in the treatment of hospitalized patients with COVID-19.

EIDD-2801

EIDD-2801 is a powerful ribonucleoside analog that inhibits the replication of RNA viruses, including SARS-CoV-2. In animal studies of two different CoV (SARS-CoV-1 and MERS), EIDD-2801 has been shown to improve respiratory function, reduce body weight loss, and reduce the amount of virus in the lung (43). Permission has been obtained from the FDA to begin clinical work by licensing the EIDD-2801 in collaboration with Ridgeback Biotherapeutics and Merck. It has 3 clinical trials (NCT04392219, NCT04405570, NCT04405739).

Anti-inflammatory Drugs and Anti-cytokine Treatments

An increase in immune reactions called "cytokine storm" and excessive production of cytokines is blamed for the rapid progression and exacerbation of COVID-19 infection. In critical COVID-19 patients, there is an excessive increase in the levels of inflammatory markers C-reactive protein and inflammatory cytokines [such as interleukin (IL)-6, TNF-a, IL-1, IL-8] in parallel with the decrease in lymphocyte count (44). In this context, the use of glucocorticoids, nonsteroidal anti-inflammatory drugs, chloroquine/hydroxychloroquine, immunosuppressive drugs, immunomodulatory drugs, and inflammatory cytokine antagonists has been considered. These include monoclonal antibodies that bind to IL-6R and antagonize its effect, TNF-alpha inhibitors, IL-1 antagonists, and janus kinase inhibitors.

Corticosteroids

In a retrospective study of 200 patients with acute respiratory distress syndrome (ARDS), a lower mortality rate was seen in patients receiving methylprednisolone (45). The number of clinical studies investigating the effects of different formulations and dosages on the use of corticosteroid drugs in COVID-19 pneumonia is constantly increasing. Following studies can be given as examples of studies investigating the effects of methylprednisolone (NCT04263402, NCT04323592, NCT04343729, NCT04273321, NCT04263402, NCT04323592, NCT04343729, NCT04273321, NCT04263402, NCT04323592, NCT04343729, NCT04273321, NCT0424354591), dexamethasone (NCT04243, NCT04331470) or dexamethasone with Siltuximab or Tacrolimus like other anti-inflammatory combination treatments (respectively NCT04329650, NCT04341038). The effect of thalidomide + low dose glucocorticoid combination on patients with severe COVID-19 is evaluated in terms of clinical efficacy (NCT04273529).

Ciclesonide

The antiviral and anti-inflammatory effects of ciclesonide, an approved corticosteroid effective in controlling chronic inflammation of the respiratory tract, is expected to be effective in the treatment of lung damage seen in severe coronavirus infections. There is a preliminary publication showing in vitro experiments that ciclesonide inhibits SARS-CoV-2 replication via viral nsp15 inhibition (46). Clinical trials have been initiated for the use of inhaled ciclesonide in COVID-19 (NCT04381364).

Interleukin-6 Antagonists

Tocilizumab is marketed as Actemra for the treatment of rheumatoid arthritis and other inflammatory conditions. In a small cohort (21 patients) retrospective study conducted in China, IL-6 receptor (IL-6R) monoclonal antibody (mAb) Tocilizumab (TCZ) developed by Genentech was able to rapidly improve clinical outcomes, normalize the fever in patients within one day, and reduce the oxygen need of 75% of the patients within five days in severe COVID-19 patients (47). There are many clinical trials (NCT04335071, NCT04306705, NCT04332094, NCT04332913) on tocilizumab, and the Chinese National Health Commission has approved its use against lung damage in patients with severe COVID-19.

Similarly, the therapeutic potential of Sarilumab, another IL-6 antagonist monoclonal antibody marketed as Kevzara for the treatment of rheumatoid arthritis, for (ARDS) in COVID-19 patients is being investigated (NCT04359901, NCT04386239, NCT04357808, NCT04324073).

Interferon Antagonists

It is thought that Emapalumab, an FDA-approved anti-IFN-γ monoclonal antibody for hemophagocytic lymphohistiocytosis, can suppress the increased cytokine production during COVID-19 infection. There is 1 clinical study investigating the efficacy and safety of its combined use with Anakinra in the treatment of COVID-19 (NCT04324021).

JAK1/2 Inhibitors

Barisitinib, a JAK1/2 inhibitor, is a licensed anti-inflammatory drug for the treatment of rheumatoid arthritis (RA). It is estimated that barisitinib may show antiviral activity by inhibiting AAK1, an important regulator of viral endocytosis (48), and there are clinical studies related to this (NCT04320277).

Ruxolitinib (Jakafi) was first approved by the FDA for the treatment of myelofibrosis, then polycythemia vera, and acute graft versus host disease in 2019. It was thought that this drug could be effective in the cytokine storm caused by COVID-19 infection by inhibiting the JAK/STAT pathway. For this reason, a clinical study has been initiated for the use of Ruxolitinib in the treatment of COVID-19 (NCT04414098).

S1P1 Inhibitors

Fingolimod is an approved S1P1 inhibitor with immunomodulatory and immunosuppressant effects. In influenza virus infection models in mice, this drug has been shown to reduce mortality and morbidity by reducing the release of proinflammatory cytokines (49). A clinical trial continues for the use of Fingolimod in COVID-19 (NCT04280588).

Colchicine

Colchicine is an immune system modulator that has long been used in the treatment of gout. Clinical trials of colchicine have been initiated in high-risk COVID-19 patients due to its potential to reduce cytokine release and inflammation and prevent complications (NCT04360980, NCT04375202, NCT04350320).

Leronlimab

ARDS seen in COVID-19 is thought to be caused by cytokine storm due to the accumulation of neutrophils in the pulmonary circulation and alveolar spaces. Leronlimab (PRO 140) can alleviate the cytokine storm seen in COVID-19 by preventing the migration of macrophages and the release of proinflammatory cytokines in the lungs. In this context, treatment with Leronlimab (CCR5 antagonist) has been shown to improve severely depleted CD8 T-lymphocyte percentages, normalize CD4/CD8 ratios, and decrease IL-6 levels in critical or terminal COVID-19 patients (50). Apart from this, there are two more clinical studies, the results of which have not yet been announced regarding the use of leronlimab in COVID-19 (NCT04347239, NCT04343651).

Medications Recommended for Lung Symptoms

Vascular endothelial growth factor (VEGF), which has been reported to have increased levels in acute lung injury (ALI) and (ARDS), is considered a potential therapeutic target in COVID-19, as it is known to cause increased vascular permeability and pulmonary edema (51).

Bevacizumab, marketed under the name Avastin for certain types of cancer, is a monoclonal antibody that inhibits VEGF. Bevacizumab was predicted to reduce pulmonary edema and mortality in ALI and ARDS treatment, and a clinical study was initiated in patients with severe COVID-19 pneumonia (NCT04275414, NCT04305106).

Drug Based Approach

The drug-based approach is based on the similarities in chemical structures of a drug not used in the target indication with the drugs used in that indication. In this approach, a new target is defined that links the drug to a new indication. The drug-based approach is based on chemical structure similarity, pharmacological action similarity, and molecular coupling between drugs (19).

Chloroquine-hydroxychloroquine

Chloroquine is a drug used in the treatment of malaria accompanied by high fever (52). Chloroquine may block SARS-CoV virus entry by altering the ACE-2 receptor and spike protein glycosylation (53). In 2003, chloroquine was found to be effective against the SARS-CoV virus that caused the SARS epidemic and was used in treatment (53). Similarly, chloroquine has been shown to effectively inhibit SARS-CoV-2 *in vitro* (34).

The effectiveness of hydroxychloroquine, a less toxic derivative of chloroquine with fewer side-effects, previously used in the treatment of patients with malaria, lupus, and arthritis, against the COVID-19 epidemic was also investigated (54). A study demonstrated that hydroxychloroquine is weakly alkaline and prevents endosomal acidification by increasing endosomal pH, thus preventing virus-target cell membrane fusion (52). Hydroxychloroquine also has anti-inflammatory and immunomodulatory effects. Hydroxychloroquine has been shown to be effective *in vitro* in SARS-CoV-2 infection (54). Following the demonstration of in vitro activity of both chloroquine and hydroxychloroquine against SARS-CoV-2, many clinical studies have been initiated in terms of its potential for use in the treatment of COVID-19 (34,54). On day six, after the inclusion of azithromycin and hydroxychloroquine combined therapy, virological improvement was observed in 100% of patients treated with the combination of hydroxychloroquine and azithromycin (57.1% in patients treated with hydroxychloroquine alone and 12.5% in the control group) (55). However, there are conflicting reports of chloroquine and hydroxychloroquine in small randomized trials and in some case reports. An article published in The Lancet magazine on May 22 demonstrated that the use of chloroquine or hydroxychloroquine in the treatment of COVID-19 increased mortality rates and caused heart rhythm disturbance. Subsequently, the article was withdrawn when serious scientific problems were revealed in the data of the study (56).

Despite all this, due to the rapid spread of COVID-19 and the increase in deaths, some countries have included chloroquine/hydroxychloroquine in their treatment protocols by evaluating the preliminary data of ongoing studies. In Turkey COVID-19 Treatment Protocol, hydroxychloroquine is recommended in all categories of COVID-19 patients, from asymptomatic to critically ill category, if the physician deems appropriate (57). Hydroxychloroquine is included in the treatment protocol for mild/moderate and critical COVID-19 patients with risk factors in Italy, the Netherlands, and Belgium (58). (NIH) COVID-19 Treatment Guidelines recommend the use of high-dose chloroquine (600 mg twice daily for ten days) to treat COVID-19 (59).

French soldiers used chloroquine as a prophylactic 100 mg per day for many years while working in areas with a high incidence of malaria. There is not enough data for the prophylactic use of chloroquine/hydroxychloroquine in COVID-19. In some treatment protocols, hydroxychloroquine can be used prophylactically to prevent asymptomatic patients from spreading the virus rapidly and protecting healthcare workers. In this context, a randomized, double-blind clinical study has been initiated, including 40,000 healthcare workers from Asia, Europe, and Africa (NCT04303507). Turkey COVID-19 Treatment Protocol stated that hydroxychloroquine can be used as 2x200 mg for 5 days in asymptomatic patients depending on the physician's choice (57).

Ivermectin

After the discovery that antimalarial chloroquine and hydroxychloroquine are effective in COVID-19, studies on another antimalarial drug, İvermectin, have accelerated. An *in vitro* study conducted at Monash University in Australia demonstrated the effectiveness of Ivermectin, an antiparasitic drug, against the SARS-CoV-2 virus. However, in order for the drug to be used in patients diagnosed with COVID-19, it is necessary to verify the effectiveness of the drug in patients with COVID-19. Therefore, more data are needed, and more clinical studies need to be concluded (60).

Azithromycin

Azithromycin is a broad-spectrum macrolide antibiotic used in the treatment of respiratory, enteric, and genitourinary system infections. In a study conducted in France, a combination of hydroxychloroquine and azithromycin was administered to 20 patients diagnosed with COVID-19 to prevent bacterial superinfection and to provide a significant reduction in viral load. According to the study, all patients recovered virologically within 6 days following treatment (55). On May 14, 2020, NIH started studies on the use of hydroxychloroquine and azithromycin in COVID-19. According to the Ministry of Health COVID-19 treatment protocol, combination therapy with azithromycin and hydroxychloroquine is recommended for patients with uncomplicated mild pneumonia and COVID-19 with severe pneumonia (57).

Conclusion and Recommendations

Drug repositioning methods save both time and cost compared to traditional drug development methods. Drug repositioning has a life-saving importance for human health by skipping some of the clinical phase tests in pandemic situations such as COVID-19 and allowing rapid access to the drug. Recent efforts to develop a treatment for COVID-19 employed drug repositioning rather than drug and target-based strategies. On the other hand, some drugs that were investigated using the disease-based approach strategy did not make a significant difference in clinical studies. Therefore, although *in vitro* data and in silico approaches are promising for rapid drug discovery in the treatment of COVID-19, the necessity of waiting for the results of clinical trials for approval of indications by drug authorities has become clearer during the COVID-19 pandemic process.

Authorship Contributions

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References

1. Park SE. Epidemiology, virology, and clinical features of severe acute respiratory syndrome -coronavirus-2 (SARS-CoV-2; Coronavirus Disease-19). Clin Exp Pediatr. 2020;63:119-24.

2. Nosengo N. Can you teach old drugs new tricks? Nature 2016;534:314-6.
3. Jourdan JP, Bureau R, Rochais C, Dallemagne P. Drug repositioning: a brief overview. J Pharm Pharmacol. 2020;72:1145-51. doi: 10.1111/jphp.13273.
4. Oprea TI, Bauman JE, Bologa CG, Buranda T, Chigaev A, Edwards BS, et al. Drug repurposing from an academic perspective. Drug Discov Today Ther Strateg 2011;8:61-9.
5. Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: a powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des 2011;7:146-57.
6. Dakshanamurthy S, Issa NT, Assefnia S, Seshasayee A, Peters OJ, Madhavan S, et al. Predicting new indications for approved drugs using a proteochemometric method. J Med Chem 2012;55:6832-48.
7. Dudley JT, Deshpande T, Butte AJ. Exploiting drug-disease relationships for computational drug repositioning. Brief Bioinform 2011;12:303-11.
8. Hodos RA, Kidd BA, Shameer K, Readhead BP, Dudley JT. In silico methods for drug repurposing and pharmacology. Wiley Interdiscip Rev Syst Biol Med 2016;8:186-210.
9. Sliwoski G, Kothiwale S, Meiler J, Lowe EW Jr. Computational methods in drug discovery. Pharmacol Rev 2014;66:334-95.
10. Kharkar PS, Warrier S, Gaud RS. Reverse docking: a powerful tool for drug repositioning and drug rescue. Future Med Chem 2014;6:333-42.
11. Lee A, Lee K, Kim D. Using reverse docking for target identification and its applications for drug discovery. Expert Opin Drug Discov 2016;11:707-15.
12. Yuan Y, Pei J, Lai L. Binding site detection and druggability prediction of protein targets for structure-based drug design. Curr Pharm Des 2013;19:2326-33.
13. Gao Z, Li H, Zhang H, Liu X, Kang L, Luo X, et al. PDTD: a web-accessible protein database for drug target identification. BMC Bioinformatics 2008;9:104.
14. Hurle MR, Yang L, Xie Q, Rajpal DK, Sanseau P, Agarwal P. Computational drug repositioning: from data to therapeutics. Clin Pharmacol Ther 2013;93:335-41.
15. Okada Y, Wu D, Trynka G, Raj T, Terao C, Ikari K, et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature 2014;506:376-81.
16. Nabirotchkin S, Peluffo AE, Rinaudo P, Yu J, Hajj R, Cohen D. Next-generation drug repurposing using human genetics and network biology. Curr Opin Pharmacol 2020;51:78-92.
17. Nelson MR, Tipney H, Painter JL, Shen J, Nicoletti P, Shen Y, et al. The support of human genetic evidence for approved drug indications. Nat Genet 2015;47:856-60.
18. Andronis C, Sharma A, Virvilis V, Deftereos S, Persidis A. Literature mining, ontologies and information visualization for drug repurposing. Brief Bioinform 2011;12:357-68.
19. Parisi D, Adasme MF, Sveshnikova A, Bolz SN, Moreau Y, Schroeder M. Drug repositioning or target repositioning: A structural perspective of drug-target-indication relationship for available repurposed drugs. Comput Struct Biotechnol J 2020;18:1043-55.
20. Wang K, Chen W, Zhou Y-S, Lian J-Q, Zhang Z, Du P, et al. SARS-CoV-2 invades host cells via a novel route: CD147-spike protein. bioRxiv 2020:2020.03.14.988345.
21. Wu R, Wang L, Kuo HD, Shannar A, Peter R, Chou PJ, et al. An Update on Current Therapeutic Drugs Treating COVID-19. Curr Pharmacol Rep 2020:1-15.
22. Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z, et al. Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. Cell 2020;181:894-904 e9.
23. Wu C, Liu Y, Yang Y, Zhang P, Zhong W, Wang Y, et al. Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. Acta Pharm Sin B 2020;10:766-88.
24. Durdagi S, Aksoydan B, Dogan B, Sahin K, Shahraki A, Birgül-İyison N. Screening of Clinically Approved and Investigation Drugs as Potential Inhibitors of SARS-CoV-2 Main Protease and Spike Receptor-Binding Domain Bound with ACE2 COVID19 Target Proteins: A Virtual Drug Repurposing Study. ChemRvix 2020. doi:10.26434/chemrxiv.12032712.v2.
25. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell 2020;181:271-80 e8.
26. Kawase M, Shirato K, van der Hoek L, Taguchi F, Matsuyama S. Simultaneous treatment of human bronchial epithelial cells with serine and cysteine protease inhibitors prevents severe acute respiratory syndrome coronavirus entry. J Virol 2012;86:6537-45.
27. Elmezayen AD, Al-Obaidi A, Sahin AT, Yelekci K. Drug repurposing for coronavirus (COVID-19): in silico screening of known drugs against coronavirus 3CL hydrolase and protease enzymes. J Biomol Struct Dyn 2020:1-13.
28. Armstrong JF, Faccenda E, Harding SD, Pawson AJ, Southan C, Sharman JL, et al. The IUPHAR/BPS Guide to PHARMACOLOGY in 2020: extending immunopharmacology content and introducing the IUPHAR/MMV Guide to MALARIA PHARMACOLOGY. Nucleic Acids Res 2020;48:D1006-D21.
29. Bagherzadeh K, Daneshvarnejad K, Abbasinazari M, Azizian H. In silico repositioning for dual inhibitor discovery of sars-cov-2 (covid-19) 3c-like protease and papain-like peptidase. Preprints 2020. doi: 10.20944/preprints202004.0084.v1.
30. Harcourt BH, Jukneliene D, Kanjanahaluethai A, Bechill J, Severson KM, Smith CM, et al. Identification of severe acute respiratory syndrome coronavirus replicase products and characterization of papain-like protease activity. J Virol 2004;7813600-12.
31. Subissi L, Imbert I, Ferron F, Collet A, Coutard B, Decroly E, et al. SARS-CoV ORF1b-encoded nonstructural proteins 12-16: replicative enzymes as antiviral targets. Antiviral Res 2014;101:122-30.
32. Simsek Yavuz S, Unal S. Antiviral treatment of COVID-19. Turk J Med Sci 2020;50:611-9.
33. Sheahan TP, Sims AC, Leist SR, Schäfer A, Won J, Brown AJ, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun 2020;11:222.

34. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 2020;30:269-71.

35. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, et al. Remdesivir for the treatment of Covid-19-preliminary report. N Eng J Med 2020;383:1813-26.

36. Nagata T, Lefor AK, Hasegawa M, Ishii M. Favipiravir: a new medication for the ebola virus disease pandemic. Disaster Med Public Health Prep2014;9:79-81.

37. Soliman EZ, Lundgren JD, Roediger MP, Duprez DA, Temesgen Z, Bickel M, et al. Boosted protease inhibitors and the electrocardiographic measures of QT and PR durations. AIDS 2011;25:367-77.

38. Scavone C, Brusco S, Bertini M, Sportiello L, Rafaniello C, Zoccoli A, et al. Current pharmacological treatments for COVID-19: What's next? British Journal of Pharmacology. 2020. doi: 10.1111/bph.15072.

39. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, et al. A Trial of Lopinavir–Ritonavir in Adults Hospitalized with Severe Covid-19. N Eng J Med 2020;382:1787-99.

40. Hung IF-N, Lung K-C, Tso EY-K, Liu R, Chung TW-H, Chu M-Y, et al. Triple combination of interferon beta-1b, lopinavir–ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. Lancet 2020;395:1695-704.

41. Pécheur E-I, Borisevich V, Halfmann P, Morrey JD, Smee DF, Prichard M, et al. The Synthetic Antiviral Drug Arbidol Inhibits Globally Prevalent Pathogenic Viruses. J Virol 2016;90:3086.

42. Blaising J, Polyak SJ, Pecheur EI. Arbidol as a broad-spectrum antiviral: an update. Antiviral Res 2014;107:84-94.

43. Sheahan TP, Sims AC, Zhou S, Graham RL, Pruijssers AJ, Agostini ML, et al. An orally bioavailable broad-spectrum antiviral inhibits SARS-CoV-2 in human airway epithelial cell cultures and multiple coronaviruses in mice. Sci Transl Med 2020;12:eabb5883.

44. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM, et al. Targeting potential drivers of COVID-19: Neutrophil extracellular traps. J Exp Med 2020;217:e20200652.

45. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, et al. Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China. JAMA Intern Med 2020;180:934-43.

46. Matsuyama S, Kawase M, Nao N, Shirato K, Ujike M, Kamitani W, et al. The inhaled corticosteroid ciclesonide blocks coronavirus RNA replication by targeting viral NSP15. bioRxiv 2020:2020.03.11.987016.

47. Xu X, Han M, Li T, Sun W, Wang D, Fu B, et al. Effective treatment of severe COVID-19 patients with tocilizumab. Proc Natl Acad Sci U S A 2020;117:10970.

48. Richardson P, Griffin I, Tucker C, Smith D, Oechsle O, Phelan A, et al. Baricitinib as potential treatment for 2019-nCoV acute respiratory disease. Lancet (London, England). 2020;395:e30-e1.

49. Walsh KB, Teijaro JR, Wilker PR, Jatzek A, Fremgen DM, Das SC, et al. Suppression of cytokine storm with a sphingosine analog provides protection against pathogenic influenza virus. Proc Natl Acad Sci U S A. 2011;108:12018-23.

50. Pattterson B, Seetthamraju H, Dhody K, Corley M, Kazempour K, Lalezari J, et al. Disruption of the CCL5/RANTES-CCR5 Pathway Restores Immune Homeostasis and Reduces Plasma Viral Load in Critical COVID-19. Research Square 2020.doi: 10.1101/2020.05.02.20084673.

51. Barratt S, Medford AR, Millar AB. Vascular endothelial growth factor in acute lung injury and acute respiratory distress syndrome. Respiration 2014;87:329-42.

52. Wu R, Wang L, Kuo H-CD, Shannar A, Peter R, Chou PJ, et al. An Update on Current Therapeutic Drugs Treating COVID-19. Curr Pharmacol Rep 2020;6:56-70.

53. Savarino A, Di Trani L, Donatelli I, Cauda R, Cassone A. New insights into the antiviral effects of chloroquine. Lancet Infect Dis 2006;6:67-9.

54. Liu J, Cao R, Xu M, Wang X, Zhang H, Hu H, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discov 2020;6:16.

55. Gautret P, Lagier J-C, Parola P, Hoang VT, Meddeb L, Mailhe M, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents 2020:105949.

56. Mehra MR, Desai SS, Ruschitzka F, Patel AN. RETRACTED: Hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis. Lancet 2020. doi:10.1016/S0140-6736(20)31180-6.

57. T.C. Sağlık Bakanlığı, Erişkin Hasta Yönetimi ve Tedavisi Rehberi. Last Accessed Date: 15.06.2020. Avaliable from: https://covid19bilgi.saglik.gov.tr/depo/tedavi/COVID19 EriskinHastaTedavisi.pdf.

58. Kamps BS, Hoffmann C. The new mini-textbook by Kamps & Hoffmann. 2nd ed. Germany: Steinhauser Verlag; 2020:115.

59. COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Last Accessed Date:16.06.2020. Available from: https://www.covid19treatmentguidelines.nih.gov/.

60. Caly L, Druce JD, Catton MG, Jans DA, Wagstaff KM. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. Antiviral Res 2020;178:104787.

Review

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COVID-19: Ivermectin; Molecular Mechanisms, Limitations, Suggestions

COVİD-19: İvermektin; Moleküler Mekanizmalar, Sınırlılıklar, Öneriler

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ABSTRACT

To date, no effective treatment has been found against coronaviruses (CoVs), which have re-emerged with severe acute respiratory syndrome CoV-2 (SARS-CoV-2) and have a high potential for disease in humans as well as domestic and wild animal species. The investigation of treatment options to combat this virus, which has a pandemic character with its high morbidity and mortality rate, is a multidisciplinary research subject. In this context, the drug repositioning has come to the agenda in the fight against SARS-CoV-2, which is the current subject. However, despite the superior characteristics of drug options other than rational usage purposes such as easy accessibility and rapid transfer to the field, the lack of evaluations for their efficacy and safety may lead to misleading. In this review, the applicability, risks, and possible molecular mechanisms of ivermectin, which is suggested to be an effective treatment option, are discussed within the framework of the pharmacokinetic and toxicokinetic properties of the drug.

Keywords: Antiviral efficacy, COVID-19, drug repurposing, ivermectin, SARS-CoV-2

ÖZ

Şiddetli akut solunum sendromu koronavirüs-2 (SARS-CoV-2) ile yeniden gündeme gelen, evcil ve yabani hayvan türlerinin yanı sıra insanlarda da hastalık yapabilme potansiyeli yüksek olan CoV karşı günümüze değin etkin bir tedavi yöntemi bulunamamıştır. Yüksek morbidite ve mortalite oranı ile pandemik bir karakter kazanan bu virüsle mücadele amacıyla tedavi seçeneklerinin araştırılması multidisipliner bir araştırma konusudur. Bu kapsamda, güncel konu niteliğinde olan SARS-CoV-2 ile mücadelede ilaçların yeniden konumlandırma çalışmaları gündeme gelmiştir. Ancak rasyonel kullanım amaçlarının dışındaki ilaç seçeneklerinin, kolay ulaşılabilirlik ve hızla uygulamaya aktarılabilir olmaları gibi üstün özelliklerine rağmen, etkinlik ve güvenilirliklerine yönelik değerlendirmelerin yapılmamış olması yanlış yönlendirmelere yol açabilmektedir. Bu derlemede, etkin bir sağaltım seçeneği olabileceği ileri sürülen ivermektinin SARS-CoV-2 tedavisinde kullanılabilirliği, riskleri ve olası moleküler mekanizmalar, ilacın farmakokinetik ve toksikokinetik özellikleri çerçevesinde ele alınmıştır.

Anahtar Sözcükler: Antiviral etkinlik, COVİD-19, ilaç yeniden konumlandırma, ivermektin, SARS-CoV-2

Introduction

Coronaviruses are single-stranded, large positive-polar RNA (having the largest genome of all RNA viruses), belonging to the order of Nidovirales. In humans, they cause respiratory tract and gastrointestinal system infections, in addition to these, mostly mild, self-healing, and rarely with severe respiratory failure and renal involvement fatal diseases. In some animal species (rats, mice, various bird species, ruminants, dogs, cats, rabbits, pigs, etc.) they cause respiratory diseases or gastroenteritis (1,2).

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Viruses belonging to the coronavirus family that are effective on humans and animals was first defined as infectious bronchitis virus in poultries in 1937, and the first human coronavirus was reported in the 1960s. The coronavirus, which has the potential to pass from animals to humans and to cause epidemics by mutation. There are 7 different coronavirus species, which infections in humans, these are CoV 229E, HCoV NL63, HCoV HKU1 and HCoV OC43, and in addition to these, SARS-CoV, Middle East respiratory syndrome (MERS) and SARS-CoV-2 (3,4). A new member of this family of viruses was identified for the first time in Wuhan, China in December 2019 and was defined as SARS-CoV-2 due to its genetic association with the virus that caused the SARS epidemic, and the disease it caused is named as by the World Health Organization as coronavirus disease (COVID-19) (5,6).

With the scope of combating this virus, which is classified as a pandemic and has become a threat to all humanity, research on vaccine development and treatment still continues, but no effective specific agent has yet been found for the treatment of coronaviruses. This situation has led to the importance of drug repositioning studies, which include the use of known drugs for new purposes, in the treatment of COVID-19 (7-10).

Current studies carried out in this context have revealed the approach that ivermectin, a compound widely used in veterinary practice for the control of parasitic infections, can be effective in the treatment of COVID-19. Ivermectin, a broad spectrum antiparasitic compound approved by the US Food and Drug Administration (FDA), is known to exhibit antiviral activity against many viruses *in vitro*. Similarly, in a study conducted in a SARS-CoV-2 infected Vero/hSLAM cell line, it was reported that an approximately 5,000-fold decrease in viral RNA was detected at the 48th hour following ivermectin administration at 5 μM concentration (11). According to the findings, the researchers stated that ivermectin has *in vitro* antiviral activity against SARS-CoV-2 and this effect is likely to occur by blocking the nuclear entry of importin alpha and beta-1 (IMPα/β1) mediated viral protein (11). Since the application of ivermectin yielded remarkable results against SARS-CoV-2, clinical studies based on the use of ivermectin + nitazoxanide combination as an adjuvant to hydroxychloroquine and azithromycin were initiated (12). In contrast, the FDA reported that such *in vitro* research is widely conducted in the early stages of drug development studies, but more research is needed on whether ivermectin is effective and safe in preventing or treating coronavirus or COVID-19 (13). When the ivermectin effectiveness in COVID-19 treatment is evaluated in relation to the pharmacokinetic profile of the drug; whenever the 5 μM concentration found effective in *in vitro* conditions adapted to *in vivo* conditions, the highest peak plasma concentration reached was 247.8 ng/mL, and this concentration was used after the approved dose (150-200 μg/kg) to reach the plasma peak concentration (30-47 ng/mL) approximately 17 times (14,15).

There are many studies evaluating the antiviral activity of ivermectin *in vitro* conditions. In a study carried out by Wagstaff et al (16), it was reported that ivermectin is an inhibitor of IMPα/β1 mediated nuclear import and prevents the replication of human immunodeficiency virus (HIV-1) by inhibiting the entry of viral proteins into the nucleus. There are also studies confirming that ivermectin is effective against Porcine Reproductive and Respiratory Syndrome Virus (17), Bovine Herpes Virus (18), Newcastle Disease Virus (19), Chikungunya Virus, Semliki Forest Virus and Sindbis Viruses (20).

In the study evaluating the activities of some molecules including ivermectin against Dengue Virus (DENV), it was reported that the *in vitro* activity test was terminated due to the high cytotoxic effect of ivermectin in the HuH-7 cell line (21). On the other hand, in a study conducted *in vitro* with liposome formulation of ivermectin, it was reported that the cytotoxic effect of the drug decreased up to 5 times, and the antiviral EC_{50} value against DENV 2 reached from 2.6 μM to 0.3 μM (22). In studies conducted at known therapeutic doses, ivermectin inhibit Pseudorabies Virus (23) in BHK-21 cell line and mice, Porcine Circovirus 2 (24) in PK-15 cell line and piglets. Ivermectin has a wide safety margin with no adverse effects up to 50 times the recommended dose in pigs (25). Remarkable findings were also obtained in studies evaluating the effectiveness of ivermectin against Flaviviruses. It has the highest potency for Yellow Fever Virus, which causes hemorrhagic fever, with an EC_{50} value of 0.5 nM. It has also proven to be a micromolar level replication inhibitor for DENV, West Nile Virus, Japanese Encephalitis Virus and Tick-borne Encephalitis Virus in *in vitro* studies (26). As a result of the application made to the World Intellectual Property Organization, it has been observed that the use of all Avermectins and Milbemicins in the treatment of Flavivirus infections is protected by patent (27).

There are many veterinary medicinal products around the world where this first endectoside, which is widely used in veterinary treatment with its powerful anthelmintic, insecticide and acaricidal effects, is included as an active ingredient (28,29). In our country, different pharmaceutical forms of ivermectins, whose marketing authorization has been issued by the Ministry of Agriculture and Forestry, General Directorate of Food and Control, has an indication area for cattle, sheep, horses and dogs (30). 1% cream form has been found by American and European authorities to be more effective and safe than known treatment options for the treatment of rose disease lesions with inflammatory lesions, known as Rosacea, and has been approved for human use (31).

There are also studies evaluating the antiviral activity of ivermectin as well as its anti-inflammatory and antitumoral activity. It has been shown that avermectin used in inflammation induced by lipopolysaccharide inhibits Tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1β production significantly and increases IL-10 production in macrophages. These effects were reported to be mediated by ERK1/2, JNK and p38 MAPK signal and activation of the NF-κB pathway (32).

Ivermectin also show antitumoral activity by effecting the MDR protein inhibition, Akt/mTOR pathway inhibition, PAK1 protein inhibition, WNT-TCF pathway inhibition, SIN3

domain inhibition, NS3 DDX23 helicase inhibition, *Nanog*/*Sox2*/*Oct4* genes downregulation, activation of P2X4/P2X and increased chlorine channel mechanisms (33).

Another effect of ivermectin is related to P-glycoprotein (P-gp). It has been observed that when the P-gp substrate ivermectin is used in combination with the P-gp inhibitor Verapamil, it can cause cytogenetic and teratogenic effects in rats (34). Similarly, when mice lacking the gene encoding P-gp (MDR1a -/-) were treated with the standard antiparasitic protocol of ivermectin, the concentration of ivermectin in brain tissue was found to be 100-fold higher than wild-type mice, and death was reported. Neurotoxic effects and hypersensitivity reactions associated with mutation in P-gp genes were observed in subpopulations of CF1 mice and coli dogs (35). In addition, since ivermectin acts as a Na^+, K^+-ATPase inhibitor at a dose of 6-17 μM IC_{50}, it has been reported that this situation indicates significant adverse effects when used at high doses (36).

There are controversial results in studies examining the genotoxicity potential of ivermectin. In this context; Ames test using *Salmonella typhimurium* and *Salmonella typhimurium* + S9, *in vitro* L5178Y/TK +/– mouse lymphoma assay, *in vitro* unprogrammed DNA synthesis in human fibroblasts, *in vitro* sister chromatid exchange in peripheral human lymphocytes and CHO-K1 cells *in vitro* in rat thymocyte It has been reported that apoptosis induction gives negative results. On the other hand, in relation to ivermectin, *in vitro* comet test, mitotic index, cell cycle progression, neutral red, MTT test results (37-39) and *in vitro* sister chromatid exchange test in peripheral human lymphocytes (40) were positively detected in CHO-K1 cells. Similarly, in their *in vivo* studies; according to the chromosomal aberration test and micronucleus test findings, they concluded that ivermectin has clastogenic and genotoxic potential in mice (41). In another study, in which the genotoxicity potential of ivermectin was evaluated with the comet test system in Zebu cattle, it was stated that the concentrations of 1% and 3.15% of the compound caused a significant DNA damage and may show genotoxic potential (42). As part of drug repositioning, a network of medicine framework has been developed over the past decade, consisting of a set of quantitative approaches and predictive tools to examine host-pathogen interactions, uncover the molecular mechanisms of infections, identify comorbidities, and quickly identify drug candidates. Gysi et al. (43), published the results that shows the drugs that may affect biological processes targeted by the virus by using *in vitro* data. In this context, it has been suggested that clinical trials of ivermectin, whose toxicity and side effects are known, should be started rapidly (43).

Studies that involve repositioning drugs, requiring less time to target with a low cost advantage, may potentially not require preclinical trials and may enter phase 2 studies directly. Combination therapy strategies can be developed that may delay or reduce resistance associated with monotherapy, thanks to known mechanisms of action. Candidate drug analogs are easily available for testing. Researchers and small-scale laboratories can be the determinant of drug positioning processes, not requiring initial investment thanks to large-scale formulation and production chains. However, due to the high doses used in in silico screening studies, toxic drugs may be initially identified as active, unforeseen and undesirable side effects may be encountered, and the effective plasma concentration may be much higher than the levels achievable for humans (44,45).

Suggestions

In silico screening and *in vitro* researches are of great importance in drug development and repositioning studies. However, as with the controversy over ivermectin, the clinical pharmacokinetics of studies in *in vitro* conditions should be carefully evaluated when adapted to *in vivo* conditions.

Ivermectin, and its derivatives are protected by patent for some indications. In the efforts to reposition the drugs that are easily accessible in the world that meet the priority needs of the population, patent protection research may be useful due to the obstacles to the public accessibility of patented substances and products.

Quality assurance systems, and good cell culture practices gain importance in terms of reliability and reproducibility of research, and it is considered important that these principles are adapted to applications by researchers. In this context, it would be useful to investigate the effectiveness of ivermectin comprehensively by different researchers.

In addition to supporting the data obtained under *in vitro* conditions with *in vivo* research, it is thought that it may be beneficial to carry out formulation development studies to increase bioavailability and reduce undesirable effects.

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References

1. İnal S. Middle East Respiratory Syndrome-Coronavirus (MERS-CoV) Enfeksiyonu: Okmeydanı Tıp Dergisi 2016;32(Ek sayı):37-45.
2. Microbiology Book Online, Viroloji Bölüm 25, Koronavirüsler, Soğuk Algınlığı ve SARS, Availeble from: https://www.microbiologybook.org/Turkish-virology/virolchapter25turk.htm
3. TÜBİTAK, Bilim ve Teknik Dergisi, Küresel Kabus, Availeble from: https://tubitak.gov.tr/sites/default/files/18842/bilim_ve_teknik_coronavirus_hakkinda.pdf
4. Hasöksüz M, Kiliç S, Saraç F. Coronaviruses and SARS-COV-2. Turkish Journal of Medical Sciences. https://doi.org/10.3906/sag-2004-127.

5. International Committee on Taxonomy of Viruses (ICTV), Naming the 2019 Coronavirus 2020. Availeble from: https://talk.ictvonline.org/
6. World Health Organisation, Naming the coronavirus disease (COVID-19) and the virus that causes it, 2020. Availeble from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/naming-the-coronavirus-disease-(covid-2019)-and-the-virus-that-causes-it
7. Pushpakom S, Iorio F, Eyers PA, Escott KJ, Hopper S, Wells A, et al. Drug repurposing: Progress, challenges and recommendations. Içinde Nat Rev Drug Discov 2018;1:41-58.
8. Information for Clinicians on Investigational Therapeutics for Patients with COVID-19 2020. Available from: https://www.cdc.gov/coronavirus/2019-ncov/hcp/therapeutic-options.html
9. T.C. Sağlık Bakanlığı COVID-19 (SARS-CoV-2 ENFEKSİYONU) REHBERİ 2020. Available from: https://covid19bilgi.saglik.gov.tr/depo/rehberler/COVID-19_Rehberi.pdf?type=file
10. World Health Organisation Off-label use of medicines for COVID-19 2020. Available from: https://www.who.int/news-room/commentaries/detail/off-label-use-of-medicines-for-covid-19
11. Caly L, Druce JD, Catton MG, Jans DA, Wagstaff KM. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. Antiviral Res 2020;178:104787. https://doi.org/10.1016/j.antiviral.2020.104787
12. U.S.National Institute of Health, Clinical Trials, 2020. Available from: https:// clinicaltrials. gov/ct2/ results?cond= COVID&term= ivermectin&cntry= &state=&city= &dist= &Search=Search
13. U.S. Food and Drug Administration, Letter to Stakeholder, 2020, Available from: https://www.fda.gov/animal-veterinary/product-safety-information/fda-letter-stakeholders-do-not-use-ivermectin-intended-animals-treatment-covid-19-humans
14. Bray M, Rayner C, Noël F, Jans D, Wagstaff K. Ivermectin and COVID-19: a report in Antiviral Research, widespread interest, an FDA warning, two letters to the editor and the authors' responses. Antiviral Res 2020;178:104805. https://doi.org/10.1016/j.antiviral.2020.104805
15. Momekov G, Momekova D. Ivermectin as a potential COVID-19 treatment from the pharmacokinetic point of view. medRxiv 2020.04.11.20061804. https://doi.org/10.1101/2020.04.11.20061804
16. Wagstaff K, Rawlinson S, Hearps A, Jans D. Novel Inhibitors of Nuclear Translocation of HIV-1 Integrase. Antiviral Res 2011;90:A48. https://doi.org/10.1016/j.antiviral.2011.03.081
17. Lee YJ, Lee C. Ivermectin inhibits porcine reproductive and respiratory syndrome virus in cultured porcine alveolar macrophages. Arch Virol 2016;161:257-68.
18. Raza S, Shahin F, Zhai W, Li H, Alvisi G, et al. Ivermectin inhibits bovine herpesvirus 1 DNA polymerase nuclear import and interferes with viral replication. Microorganisms 2020;8:1-15.
19. Azeem S, Ashraf M, Rasheed MA, Anjum AA, Hameed. Evaluation of cytotoxicity and antiviral activity of ivermectin against Newcastle disease virus. Pak J Pharm Sci 2015;28:597-602.
20. Varghese FS, Kaukinen P, Gläsker S, Bespalov M, Hanski L, Wennerberg. Discovery of berberine, abamectin and ivermectin as antivirals against chikungunya and other alphaviruses. Antiviral Res 2016;126:117-24.
21. Chu JJH, Lee RCH, Ang MJY, Wang WL, Lim HA, Wee JLK, et al. Antiviral activities of 15 dengue NS2B-NS3 protease inhibitors using a human cell-based viral quantification assay. Antiviral Res 2015;118:68-74.
22. Croci R, Bottaro E, Chan KWK, Watanabe S, Pezzullo M, Mastrangelo E, et al. Liposomal Systems as Nanocarriers for the Antiviral Agent Ivermectin. Int J Biomater 2016. https://doi.org/10.1155/2016/8043983
23. Lv C, Liu W, Wang B, Dang R, Qiu L, Ren J, et al. Ivermectin inhibits DNA polymerase UL42 of pseudorabies virus entrance into the nucleus and proliferation of the virus in vitro and vivo. Antiviral Res 2018;159:55-62.
24. Wang X, Lv C, Ji X, Wang B, Qiu L, Yang Z. Ivermectin treatment inhibits the replication of Porcine circovirus 2 (PCV2) in vitro and mitigates the impact of viral infection in piglets. Virus Res 2019;263:80-6.
25. Sanford SE, Rehmtulla AJ, Josephson GKA. Ivermectin overdose and toxicosis in neonatal pigs. Can Vet J 1998;29:735-36.
26. Mastrangelo E, Pezzullo M, De burghgraeve T, Kaptein S, Pastorino B, Dallmeier K, et al. Ivermectin is a potent inhibitor of flavivirus replication specifically targeting NS3 helicase activity: New prospects for an old drug. J Antimicrob Chemother 2012;67:1884-94.
27. World Intellectul Property Organisation (WIPO). 2020. WO2011051159 - Avermectıns and mılbemycıns for the treatment of flavıvırus ınfectıons. Available from: https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2011051159&recNum=29&docAn=EP2010065880&queryString=DORAMECTIN&maxRec=452
28. U.S.Food And Drug Administration (FDA), (2018). Available from: https://animaldrugsatfda.fda.gov/adafda/views/#/search
29. European Medicines Agency (EMA), (2018). Available from: http://www.eudrapharm.eu/eudrapharm/searchbykeywordresult.do
30. Tarım ve Orman Bakanlığı, (2020). Ruhsatlı Veteriner Tıbbi Ürünler, Available from: https://vtu.tarim.gov.tr/FYerli.aspx.
31. Crump A. Ivermectin: Enigmatic multifaceted "wonder" drug continues to surprise and exceed expectations. J Antibiot (Tokyo) 2017;70:495-505.
32. Ci X, Li H, Yu Q, Zhang X, Yu L, Chen N, et al. Avermectin exerts anti-inflammatory effect by downregulating the nuclear transcription factor kappa-B and mitogen-activated protein kinase activation pathway. Fund Clin Pharmacol 2009;23:449-55.
33. Juarez M, Schcolnik-Cabrera A, Dueñas-Gonzalez A. The multitargeted drug ivermectin: from an antiparasitic agent to a repositioned cancer drug. Am J Cancer Res 2018;8:317-31.
34. El-Ashmawy IM, El-Nahas AF, Bayad AE. Teratogenic and cytogenetic effects of ivermectin and its interaction with P-glycoprotein inhibitor. Res Vet Sci 2011;90:116-23.
35. Schinkel AH, Smit JJ, Van Tellingen O, Beijnen JH, Wagenaar E, Van Deemter L, et al. Disruption of the mouse mdr1a P-glycoprotein

gene leads to a deficiency in the blood–brain barrier and to increased sensitivity to drugs. Cell 1994;77:491-502.

36. Pimenta PHC, Silva CLM, Noel F. Ivermectin is a nonselective inhibitor of mammalian P-type ATPases. Naunyn-Schmiedeberg's Arch Pharmacol 2010;381:147-52.
37. EMA, (2004). Committee For Medicinal Products For Veterinary Use EMEA/MRL/915/04-Final Ivermectin (Modification of Maximum Residue Limits) Summary Report (5).
38. Molinari G, Soloneski S, Reigosa MA, Larramendy ML. In vitro genotoxic and cytotoxic effects of ivermectin and its formulation ivomec® on Chinese hamster ovary (CHOK1) cells. J Hazard Mater 2009;165:1074-82.
39. Molinari G, Soloneski, S, Larramendy ML. New ventures in the genotoxic and cytotoxic effects of macrocyclic lactones, Abamectin and Ivermectin. Cytogenet Genome Res 2010;128:37-45.
40. Aleksić N, Barjaktarović N. Investigation on sister chromatid exchange (SCE) by ivermectin. Genetika 1993;25:219-25.
41. Sweify KM, Abd I, Darwish EM, Demerdash D, El A, Hafez M. The cytogenetic potential of ivermectin on bone marrow cells of mice in vivo. OSR-JESTFT, 2015;9:2319-99.
42. Montes V, De La Ossa VJ, Pérez Cordero A. Comet assay to determine genetic damage by the use of ivermectin in zebu cows (Bos taurus indicus). Revista MVZ Córdoba 2017;22:5959-65.
43. Gysi DM, Do Valle I, Zitnik M, Ameli A, Gan X, Varol O, et al. Network Medicine Framework for Identifying Drug Repurposing Opportunities for COVID-19. ArXiv 2020;arXiv:2004.07229v1. Preprint.
44. Mercorelli B, Palù G, Loregian A. Drug Repurposing for Viral Infectious Diseases: How Far Are We? Trends Microbiol 2018;26:865-76.
45. García-Serradilla M, Risco C, Pacheco B. Drug repurposing for new, efficient, broad spectrum antivirals. Virus Res 2019;264:22-31.

Review

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An Overview of COVID-19 Medicines in Current Guidelines

Güncel Kılavuzlardaki COVİD-19 İlaçlarına Bir Bakış

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ABSTRACT

An acute respiratory disease caused by a new coronavirus (Severe acute respirotary syndrome-coronavirüs-2, previously known as 2019-nCoV), coronavirus disease 2019 (COVID-19), appeared in December 2019 and then has spread rapidly throughout the world starting from China, Japan and South Korea. As of January 30, 2020, the World Health Organization has officially declared the COVID-19 outbreak. Considering the clinical symptoms of COVID-19, it has many symptoms such as high fever, cough, and fatigue. It is reported that this disease is very severe and causes serious consequences such as cytokine storm and acute respiratory distress syndrome in the elderly and those with chronic diseases. Currently, scientists are trying to find a specific antiviral treatment strategy. Various medications such as hydroxychloroquine, lopinavir/ritonavir, ribavirin, remdesivir and favipiravir are currently being applied in clinical trials to test their efficacy and safety worldwide in COVID-19 treatment, and some promising results have been achieved so far. In this review, agents with potential efficacy against COVID-19 are presented in summary.

Keywords: COVID-19, antivirals, treatment, SARS-CoV-2

ÖZ

Yeni bir koronavirüsün (daha önce 2019-nCoV olarak bilinen Şiddetli akut solunum enfeksiyonu-koronavirüs-2) neden olduğu akut solunum yolu hastalığı, koronavirüs hastalığı 2019 (COVİD-19) Aralık 2019'da ortaya çıkmış ve daha sonra Çin, Japonya ve Güney Kore'den başlayarak Dünya genelinde hızlı bir yayılım göstermiştir. 30 Ocak 2020 itibariyle de Dünya Sağlık Örgütü COVİD-19 salgınını resmi olarak ilan etmiştir. COVİD-19'un klinik semptomlarına bakıldığında yüksek ateş, öksürük, yorgunluk gibi birçok semptomu vardır. Yaşlılarda ve kronik hastalıkları olanlarda bu hastalığın çok ağır geçtiği ve sitokin fırtınası ile akut solunum sıkıntısı sendromu (ARDS) gibi ciddi sonuçlara neden olduğu bildirilmektedir. Şu anda bilim insanları tarafından spesifik antiviral tedavi stratejisi bulunmaya çalışılmaktadır. Hidroksiklorokin, lopinavir/ritonavir, ribavirin, remdesivir ve favipiravir gibi çeşitli ilaçlar şu anda Dünya çapında COVİD-19 tedavisinde etkinliklerini ve güvenliklerini test etmek için klinik çalışmalarda uygulanmaktadır ve şimdiye kadar bazı umut verici sonuçlar elde edilmiştir. Bu derlemede, COVİD-19'a karşı potansiyel etkinliği olan ajanlar özet halinde sunulmaktadır.

Anahtar Sözcükler: COVID-19, antiviraller, tedavi, SARS-CoV-2

Introduction

The Severe acute respirotary syndrome-coronavirus-2 virus (formerly 2019-nCoV) appeared in Wuhan, Hubei province of China in December 2019, and then spread very rapidly across the world, starting from China, Japan and South Korea. To date, more than 3 million cases and more than 200 thousand deaths have been reported worldwide (1). The first COVID-19 case was confirmed in Turkey on March 10, 2020 and a total of 107,773 cases were approved as of April 25th 2020, and it was reported that a total of 2,706 patients died (2). Considering the lack of effective antiviral therapy against COVID-19, current treatments

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are predominantly for symptoms (3). That is why, scientists have recently intensified their work rapidly on drug therapy for COVID-19 treatment. We can use some treatment strategies against coronaviruses based on the experience of struggling in SARS-CoV and Middle East Respiratory Syndrome corona virus (MERS-CoV) outbreaks (4). Some agents have been quickly tested in clinical trials and have been reported to show activity against COVID-19 (5). The summary of current adult and pediatric patient medication guideline published by the Republic of Turkey Ministry of Health dated April 14, 2020 is shown in

Table 1. Current guideline for adult and child patient drug treatment

Name of drug	Dosage	Administration way	Time
Treatment recommendations for asymptomatic definite COVID-19 cases to be monitored as outpatient and for uncomplicated patients or patients with mild pneumonia-possible COVID-19			
Hydroxychloroquine	2x200 mg	Oral	5 days
Recommendations for COVID-19 Patients with indication for hospitalization			
Treatment in uncomplicated possible/definitely diagnosed COVID-19 Cases			
Hydroxychloroquine	2x200 mg	Oral	5 days
-/+ Azithromycin	1st day 500 mg +250 mg x4 days	Oral	5 days
Treatment in possible/definitely diagnosed COVID-19 cases with severe pneumonia			
Hydroxychloroquine	2x400 mg loading dose +2x200 mg	Oral	5 days
-/+ Favipiravir	2x1,600 mg loading dose +2x600 mg maintenance dose	Oral	5 days
-/+ Azithromycin	1st day 500 mg +250 mg x4 days	Oral	5 days
Treatment in patients whose pneumonia findings have progressed while receiving hydroxychloroquine treatment			
Favipiravir (Hydroxychloroquine treatment should be completed within 10 days and stopped)	2x1,600 mg loading dose +2x600 mg maintenance dose	Oral	5 days
Treatment in pregnants with the definite diagnosis of COVID-19			
Hydroxychloroquine	2x200 mg	Oral	5 days
-/+ Lopinavir 200 mg/ Ritonavir 50 mg	200 mg/50 mg twice a day	Oral	10-14 days
Dosages and administration ways of drugs that can be used in the treatment of children			
Hydroxychloroquine	6.5 mg/kg/dose of Hydroxychloroquine for twice on the first day; maximum dose on the first day: 400 mg/dose; on the 2nd-5th days, 3.25 mg/kg/dose of Hydroxychloroquine for twice a day: maximum dose 200 mg/dose	Oral	5 days
-/+ Azithromycin	**1-5 month children** 10 mg/kg/dose (max dose 500 mg/dose) **>6 month children and adolescents** 10 mg/kg single dose on the first day (max dose 500 mg/dose), Then, continuing with 5 mg/kg single dose a day for 2-5 days (max dose 250 mg/dose) totally 5 days	Oral	5days
-/+ Lopinavir 200 mg/ Ritonavir 50 mg	**Between 14 days-6 months:** Lopinavir 16 mg/kg PO BID **Between 6 month-old-18 year-old:** 15-25 kg: 200 mg-50 mg PO BID 26-35 kg: 300 mg-75 mg PO BID >35 kg: 400 mg-100 mg PO BID	Oral	10-14 days

COVID-19: Coronavirus disease 2019, max: Maximum

Table 1 (6,7). In the most current version of the guideline, the use of antimicrobials such as hydroxychloroquine lopinavir/ritonavir, azithromycin and favipiravir is recommended for the treatment of COVID-19 (Table 2).

Hydroxychloroquine is an antimalarial widely used in malaria, which was discovered to be a potential broad-spectrum antiviral in 2006 (8,9). Although the mechanism of action of hydroxychloroquine is not fully understood, it appears to block viral entry into cells by inhibiting the glycosylation of host receptors, proteolytic processing and endosomal acidification. This drug has also been shown to suppress cytokine production, such as interleukin (IL)-6 and tumor necrosis factor-α, and has immunomodulatory effects in host cells through the inhibition of lysosomal activity and autophagy (10,11). Chloroquine has the ability to inhibit replication cycles against human immunodeficiency virus (HIV) and other inflammation-related viruses (12).

Hydroxychloroquine has blocked SARS-CoV-2 infection at a low micromolar concentration with a semi-maximum effective concentration (EC_{50}) of 1.13 μM and a semi-cytotoxic concentration (CC_{50}) greater than 100 μM (13). Currently, there are some randomized clinical trials that examine their role in COVID-19 therapy (14). Chloroquine is known to inhibit pH dependent steps of replication of various viruses (11). Hydroxychloroquine studies are planned for post-exposure prophylaxis after chloroquine prophylaxis (NCT04303507) and high-risk exposures (NCT04308668) in healthcare professionals (15). Although the antiviral properties of chloroquine treat people with the disease, it should be remembered that this will change depending on the disease, chloroquine concentration and duration of treatment (16). Chloroquine is still the first drug that comes to mind because it leads to rapid fever reduction and an immediate improvement in lung computed tomography findings, and at least 10 clinical studies are currently being performed (17).

Scientists have used protease inhibitors of lopinavir and ritonavir along with other drugs to treat HIV-1-infected adults and children over the age of 14 years (18) and HIV-infected people (19). Chu et al. (20) have confirmed in *in vitro* and clinical

Table 2. Potential antivirals used in the treatment process of COVID-19

Name of Drug	General definition	Mechanism of action	Targeted diseases	Reference
Lopinavir/ritonavir	Protease inhibitor	Inhibition of HIV-1 protease for protein cleavage leading to non-infectious, immature viral particles	HIV/AIDS, SARS, MERS	(19-21)
Chloroquine	9-aminoquinoline	Increased endosomal pH, immunomodulator, autophagy inhibitors	Malaria, autoimmune diseases	(11,13,14,41)
Remdesivir (GS-5734)	Nucleotide analog prodrug	Interaction after entry of the virus into the host	Ebola, SARS, MERS	(42-44)
Nafamostat	Synthetic serine protease inhibitor	It prevents membrane fusion by reducing cathepsin B release; anticoagulant activities	Influenza, MERS, Ebola	(45,46)
Ribavirin	Synthetic guanosine nucleoside	Interfering with viral mRNA synthesis (a broad-spectrum activity against several RNA and DNA viruses)	HCV, SARS, MERS	(46-48)
Oseltamivir	Neuroaminidase inhibitor	Inhibiting the activity of the viral neuraminidase enzyme, preventing proliferation from the host cell, viral replication and infectivity	Influenza, viruses A	(49,50)
Penciclovir/acyclovir	Nucleoside analog	A synthetic acyclic guanine derivative resulting in chain termination	HSV, VZV	(51)
Ganciclovir	Nucleoside analog	Powerful inhibitor of the Herpesvirus family, including cytomegalovirus	AIDS-related cytomegalovirus infections	(52)
Favipiravir (T-705)	Nucleoside analog: Viral RNA Polimerase inhibitor	Influencing viral genetic replication to prevent replication without affecting host cellular RNA or DNA synthesis	Ebola, Influenza, A(H1N1)	(53-55)
Nitazoxanide	Antiprotozoal agent	Modulation of the survival, growth and proliferation of a range of extracellular and intracellular protozoa, helminths, anaerobic and microaerophilic bacteria, viruses	A wide range of viruses, including human/animal coronaviruses	(56-58)

studies that lopinavir/ritonavir has anti-SARS-CoV activity and Arabi et al. (21) have confirmed that they have anti-MERS-CoV activity. They also have demonstrated that they can cure patients.

Early reports of lopinavir/ritonavir for the treatment of COVID-19 are mostly case reports and small-scale retrospective, non-randomized cohort studies, which makes it difficult to detect the direct treatment effect of lopinavir/ritonavir. More recently, Cao et al. (22) have reported open-label randomized clinical trial results comparing the efficacy of lopinavir/ritonavir with standard care in 199 patients with COVID-19. A study conducted in South Korea reported that the viral load of a COVID-19 positive patient decreased with lopinavir/ritonavir treatment (23).

Favipiravir is currently one of the drugs that have undergone clinical trials for the treatment of COVID-19. Favipiravir is a new RNA-dependent RNA polymerase inhibitor. Favipiravir is converted into an active phosphoribosyl form (favipiravir-RTP) in cells and is recognized by the viral RNA polymerase as a substrate, thereby inhibits RNA polymerase activity (24). Therefore, favipiravir is thought to have a potential antiviral effect on SARS-CoV-2, which is an RNA virus. There are a limited number of clinical trials that support the use of favipiravir for COVID-19. In a prospective, randomized, multicenter study, favipiravir (n=120) was compared with Arbidol (n=120) for the treatment of moderate and severe COVID-19 infections. On the 7th day, clinical improvement differences were observed in patients with moderate infection (71.4% favipiravir and 55.9% arbidol, p=0.019). No significant difference was observed in the severe or severe and moderate (combined) arms (25). In another study conducted in China, considering the first results of a total of 80 patients (including the experimental group and the control group), favipiravir was shown to have a stronger antiviral effect than lopinavir/ritonavirin (26).

Azithromycin is an antibiotic from the macrolide group with a wide range of uses, especially for the treatment of gram-positive cocci. Azithromycin has been shown to be active *in vitro* against Zika and Ebola viruses and to prevent severe respiratory infections when administered to patients who have had a viral infection (27-29). Another study reported that the combination of azithromycin-hydroxychloroquine (6/6, 100%) in 6 COVID-19 patients resulted in numerically superior viral clearance compared to hydroxychloroquine monotherapy (8/14, 57%) (30).

In addition to the medicines in the above guidelines, a few more medicines attract attention. Of these, remdesivir, which was developed for Ebola, is an antiviral drug with a nucleoside analog and broad-spectrum anti-RNA (31) and shows broad-spectrum antiviral activity against several RNA viruses. Animal experiments (32) have shown that remdesivir can effectively reduce viral load in lung tissue of MERS-CoV-2-infected mice, improve lung function, and alleviate pathological damage in lung tissue. Based on data collected in the *in vitro* mouse cell culture model, remdesivir has been found to affect NSP12 polymerase in coronaviruses (23). Wang et al. (13) found that remdesivir strongly prevents SARS-CoV-2 infection at low micromolar concentrations and has a high selectivity index. Holshue et al. (31) reported that remdesivir gave promising results in the treatment of a patient with COVID-19 in the USA. The combination of remdesivir and chloroquine has been proven to effectively inhibit the recently occurring SARS-CoV-2 as *in vitro*. Currently, randomized, placebo-controlled, double-blind phase III studies are being conducted on 761 patients in many hospitals in Wuhan, the first place of outbreak. The results of the trials are expected to be announced in the next few weeks (33). An *in vitro* activity of oseltamivir, another neuraminidase inhibitor, which is normally approved for influenza treatment, has not been documented against SARS-CoV-2. In China, the COVID-19 outbreak initially occurred during the peak influenza season, so a large number of patients underwent empirical oseltamivir therapy, not as a therapeutic intervention, until SARS-CoV-2 was discovered (34). In current guidelines, it is added to the treatment in viral pneumonia seen with COVID-19 (35). Ribavirin is also one of the new drugs added to the guidelines. It is a broad spectrum nucleoside analogue with antiviral effects. One study compared 111 patients with severe acute respiratory syndrome (SARS) treated with ribavirin and 41 SARS patients treated in combination with lopinavir/ritonavir and ribavirin; Acute respiratory distress syndrome (ARDS) and mortality risk were found to be lower in patients treated with combined therapy (20). However, ribavirin was found to have limited *in vitro* activity against SARS-CoV and was shown to require high concentrations to inhibit viral replication, which led to high dose and combination therapy (36). It is thought to have limited use for COVID-19 treatment due to its efficacy data and toxicity without ribavirin for other nCoV types (5). These drugs require more clinical evidence before they are recommended. Other drugs recommended for treatment are arbidol (an antiviral drug available in Russia and China), intravenous immunoglobulin, interferons, and plasma of patients recovered from COVID-19 (37-39). Among the candidate drugs to treat COVID-19, repositioning old drugs for use as antiviral therapy is an interesting strategy because information on safety profile, side effects, posology, and drug interactions is well known (40).

Discussion

For COVID-19, which started in December 2019 and spread all over the world, scientists have made great progress in the characterization of the virus, and vaccine-drug research that can actively be effective in combating COVID-19 has also accelerated. There is currently no proven antiviral specific for COVID-19. Other drugs recommended for treatment are arbidol (an antiviral drug available in Russia and China), intravenous immunoglobulin, interferons, and plasma of patients recovered from COVID-19 (37-39). Among the candidate drugs to treat COVID-19, repositioning old drugs for use as antiviral therapy is an interesting strategy because information on safety profile, side effects, posology, and drug interactions is well known (40). This indicates that although a pandemic is present, it is necessary to continue the work against the same pandemic with increasing difficulties. At present, more data are needed

to further demonstrate the effectiveness of antiviral treatments against the virus. In addition, studies are needed to investigate the transmission and pathogenicity mechanisms that need to be revealed. The most important issue is to reveal the molecular mechanism of viral entry and viral replication for targeted vaccine-drug studies.

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References

1. Center JHCR. COVID-19 Map 2020. Available from: https://coronavirus.jhu.edu/map.html.
2. T.C. Sağlık Bakanlığı. T.C Sağlık Bakanlığı Korona Tablosu 2020. Available from: https://covid19.saglik.gov.tr/
3. Wang Z, Chen X, Lu Y, Chen F, Zhang W. Clinical characteristics and therapeutic procedure for four cases with 2019 novel coronavirus pneumonia receiving combined Chinese and Western medicine treatment. Biosci Trends 2020;14:64-8.
4. Zumla A, Chan JF, Azhar EI, Hui DS, Yuen K-Y. Coronaviruses-drug discovery and therapeutic options. Nature reviews Drug Discov 2016;15:327.
5. Sanders JM, Monogue ML, Jodlowski TZ, Cutrell JB. Pharmacologic treatments for coronavirus disease 2019 (COVID-19): a Review. JAMA 2020;323:1824-36.
6. T.C. Sağlık Bakanlığı. COVID-19 Erişkin Hasta Tedavisi. 2020. Available from: https://covid19.saglik.gov.tr/TR-66926/eriskin-hasta-tedavisi.html p. 1-19.
7. T.C. Sağlık Bakanlığı. COVID-19. Çocuk Hasta Yönetimi ve Tedavisi. Available from: https://covid19.saglik.gov.tr/Eklenti/38596/0/covid-19rehbericocukhastayonetimivetedavipdf.pdf 2020. p. 1-5.
8. Savarino A, Di Trani L, Donatelli I, Cauda R, Cassone A. New insights into the antiviral effects of chloroquine. Lancet Infect Dis 2006;6:67-9.
9. Aguiar AC, Murce E, Cortopassi WA, Pimentel AS, Almeida MM, Barros DC, et al. Chloroquine analogs as antimalarial candidates with potent in vitro and in vivo activity. Int J Parasitol Drugs Drug Resist 2018;8:459-64.
10. Zhou D, Dai S-M, Tong Q. COVID-19: a recommendation to examine the effect of hydroxychloroquine in preventing infection and progression. J Antimicrob Chemother 2020;75:1667-70.
11. Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, et al. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. J Virol 2005;2:69.
12. Boelaert JR, Piette J, Sperber K. The potential place of chloroquine in the treatment of HIV-1-infected patients. J Clin Virol 2001;20:137-40.
13. Wang L. Cell Res. the press; 2019.
14. Savarino A, Boelaert JR, Cassone A, Majori G, Cauda R. Effects of chloroquine on viral infections: an old drug against today's diseases. Lancet Infect Dis 2003;3:722-7.
15. ClinicalTrials.gov is a database of privately and publicly funded clinical studies conducted around the world. Avaialble from: ClinicalTrials.gov. Home 2020.
16. Devaux CA, Rolain J-M, Colson P, Raoult D. New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? Int J Antimicrob Agents 2020:105938.
17. Harrison C. Coronavirus puts drug repurposing on the fast track. Nat Biotechnol 2020;38:379-81.
18. Su B, Wang Y, Zhou R, Jiang T, Zhang H, Li Z, et al. Efficacy and tolerability of lopinavir/ritonavir-and efavirenz-based initial antiretroviral therapy in HIV-1-infected patients in a tertiary care hospital in Beijing, China. Front Pharmacol 2019;10:1472.
19. Cvetkovic RS, Goa KL. Lopinavir/ritonavir. Drugs 2003;63:769-802.
20. Chu C, Cheng V, Hung I, Wong M, Chan K, Chan K, et al. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. Thorax 2004;59:252-6.
21. Arabi YM, Alothman A, Balkhy HH, Al-Dawood A, AlJohani S, Al Harbi S, et al. Treatment of Middle East Respiratory Syndrome with a combination of lopinavir-ritonavir and interferon-β1b (MIRACLE trial): study protocol for a randomized controlled trial. Trials 2018;19:81.
22. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, et al. A trial of lopinavir–ritonavir in adults hospitalized with severe Covid-19. N Eng J Med 2020;382:1787-99.
23. Lim J, Jeon S, Shin H-Y, Kim MJ, Seong YM, Lee WJ, et al. Case of the index patient who caused tertiary transmission of COVID-19 infection in Korea: the application of lopinavir/ritonavir for the treatment of COVID-19 infected pneumonia monitored by quantitative RT-PCR. J Korean Med Sci 2020;35:e79.
24. Furuta Y, Komeno T, Nakamura T. Favipiravir (T-705), a broad spectrum inhibitor of viral RNA polymerase. Proc Jpn Acad Ser B Phys Biol Sci 2017;93:449-63.
25. Chen C, Huang J, Cheng Z, Wu J, Chen S, Zhang Y, et al. Favipiravir versus Arbidol for COVID-19: a randomized clinical trial. MedRxiv. 2020.
26. Dong L, Hu S, Gao J. Discovering drugs to treat coronavirus disease 2019 (COVID-19). Drug Discover Ther 2020;14:58-60.
27. Bosseboeuf E, Aubry M, Nhan T, Pina J, Rolain J, Raoult D, et al. Azithromycin inhibits the replication of zika virus. J Antivir Antiretrovir 2018;10:6-11.
28. Madrid PB, Panchal RG, Warren TK, Shurtleff AC, Endsley AN, Green CE, et al. Evaluation of Ebola virus inhibitors for drug repurposing. ACS Infect Dis 2015;1:317-26.
29. Bacharier L, Guilbert T, Mauger D, Boehmer S, Beigelman A, Fitzpatrick A, et al. National Heart, Lung, and Blood Institute's

AsthmaNet. Early administration of azithromycin and prevention of severe lower respiratory tract illnesses in preschool children with a history of such illnesses: a randomized clinical trial. JAMA 2015;314:2034-44.

30. Gautret P, Lagier J-C, Parola P, Meddeb L, Mailhe M, Doudier B, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrobi Agents 2020:105949.
31. ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, et al. First case of 2019 novel coronavirus in the United States. N Eng J Med 2020;382:929-36.
32. Sheahan TP, Sims AC, Leist SR, Schäfer A, Won J, Brown AJ, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun 2020;11:1-14.
33. Arena C. Coronavirus outbreak: Top coronavirus drugs and vaccines in development. Last Accessed Date: 26.03.2020. Available from: https://www.clinicaltrialsarena.com/analysis/coronavirus-mers-cov-drugs.
34. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. JAMA 2020;323:1061-9.
35. Türkiye Ulusal Alerji ve Klinik İmmünoloji Derneği. COVID-19 Tedavisinde Kullanılan İlaçlara Gelişen İstenmeyen İlaç Reaksiyonları 2020 Last Accessed Date: 26.03.2020. Avaialable from: https://www.aid.org.tr/covid-19-tedavisinde-kullanilan-ilaclara-gelisen-istenmeyen-ilac-reaksiyonlari/
36. Stockman LJ, Bellamy R, Garner P. SARS: systematic review of treatment effects. PLoS Med 2006;3:e343.
37. Jin Y-H, Cai L, Cheng Z-S, Cheng H, Deng T, Fan Y-P, et al. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). Mil Med Res 2020;7:4.
38. Zhang L, Liu Y. Potential interventions for novel coronavirus in China: a systemic review. Journal of medical virology. 2020.
39. Jie Z, He H, Xi H, Zhi Z. Multicenter Collaboration Group of Department of Science and Technology of Guangdong Province and Health Commission of Guangdong Province for Chloroquine in the Treatment of Novel Coronavirus Pneumonia. Expert Consensus on Chloroquine Phosphate for the Treatment of Novel Coronavirus Pneumonia [in Chinese]. Zhonghua Jie He He Hu Xi Za Zhi 2020;43:185-8.
40. Colson P, Rolain J-M, Lagier J-C, Brouqui P, Raoult D. Chloroquine and hydroxychloroquine as available weapons to fight COVID-19. Int J Antimicrob Agents 2020;105932(10.1016).
41. Golden EB, Cho H-Y, Hofman FM, Louie SG, Schönthal AH, Chen TC. Quinoline-based antimalarial drugs: a novel class of autophagy inhibitors. Neurosurg Focus 2015;38:E12.
42. Agostini ML, Andres EL, Sims AC, Graham RL, Sheahan TP, Lu X, et al. Coronavirus susceptibility to the antiviral remdesivir (GS-5734) is mediated by the viral polymerase and the proofreading exoribonuclease. mBio 2018;9:e00221-18.
43. Tchesnokov EP, Feng JY, Porter DP, Götte M. Mechanism of inhibition of Ebola virus RNA-dependent RNA polymerase by remdesivir. Viruses 2019;11:326.
44. Lo MK, Feldmann F, Gary JM, Jordan R, Bannister R, Cronin J, et al. Remdesivir (GS-5734) protects African green monkeys from Nipah virus challenge. Sci Transl Med 2019;11:eaau9242.
45. Hsieh H-P, Hsu JT-A. Strategies of development of antiviral agents directed against influenza virus replication. Curr Pharm Des 2007;13:3531-42.
46. Hepatitis C guidance 2018 update: AASLD-IDSA recommendations for testing, managing, and treating hepatitis C virus infection. Clin Infect Dis 2018;67:1477-92.
47. Tsang K, Zhong NS. SARS: pharmacotherapy. Respirology 2003;8:S25-30.
48. Arabi YM, Shalhoub S, Mandourah Y, Al-Hameed F, Al-Omari A, Al Qasim E, et al. Ribavirin and interferon therapy for critically ill patients with middle east respiratory syndrome: a multicenter observational study. Clini Infect Dis 2020;70:1837-44.
49. McQuade B, Blair M. Influenza treatment with oseltamivir outside of labeled recommendations. Am J Health System Pharm 2015;72:112-6.
50. Jefferson T, Jones M, Doshi P, Spencer EA, Onakpoya I, Heneghan CJ. Oseltamivir for influenza in adults and children: systematic review of clinical study reports and summary of regulatory comments. BMJ 2014;348:g2545.
51. Shiraki K. Antiviral drugs against alphaherpesvirus. Human Herpesviruses: Springer; 2018:103-22.
52. Guo Y-R, Cao Q-D, Hong Z-S, Tan Y-Y, Chen S-D, Jin H-J, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak–an update on the status. Mil Med Res 2020;7:1-10.
53. Furuta Y, Gowen BB, Takahashi K, Shiraki K, Smee DF, Barnard DL. Favipiravir (T-705), a novel viral RNA polymerase inhibitor. Antiviral Res 2013;100:446-54.
54. Goldhill DH, te Velthuis AJ, Fletcher RA, Langat P, Zambon M, Lackenby A, et al. The mechanism of resistance to favipiravir in influenza. Proc Natl Acad Sci U S A 2018;115:11613-8.
55. Cardile AP, Warren TK, Martins KA, Reisler RB, Bavari S. Will there be a cure for Ebola? Ann Rev Pharmacol Toxicol 2017;57:329-48.
56. Rossignol J-F. Nitazoxanide: a first-in-class broad-spectrum antiviral agent. Antiviral Res 2014;110:94-103.
57. Cao J, Forrest JC, Zhang X. A screen of the NIH Clinical Collection small molecule library identifies potential anti-coronavirus drugs. Antiviral Res 2015;114:1-10.
58. Rossignol J-F. Nitazoxanide, a new drug candidate for the treatment of Middle East respiratory syndrome coronavirus. J Infect Public Health 2016;9:227-30.

Review

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Current Therapeutic Interventions for COVID-19

Güncel Bilgiler Işığında COVİD-19'da İlaç Tedavisi

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ABSTRACT

Severe acute respiratory syndrome coronavirus-2 is an important infectious agent that was first detected in China, causing the pandemic and death of thousands of people. Although the clinic of this disease, called coronavirus disease-19 (COVID-19), is variable, cytokine storm and different mechanisms can cause increased mortality as a result of progressive and serious clinical presentations. Since it belongs to a well-known group of viruses, researchers have gained momentum after the isolation and the identification of the features of the virus. Although specific drugs began to be developed, the first potential candidate drugs were drugs such as broad-spectrum antibiotics, antiviral agents, anti-parasitic agents, and interferon, which were planned to be used with similar indications before pandemic. Trials on all steps such as prophylactic and supporting therapies, as well as care for critically ill patients and vaccine investigation studies are still ongoing. The drugs used in the light of the guidelines were frequently updated and some changes were made as a result of reports on side effects and efficacy evaluations. Especially in some drug combinations, side effects like prolonged QT interval, drug-drug interactions, and restrictions on the use of some drugs in the pediatric age group or pregnancy limit the specific, evidence-based and reliable treatment. Although there are many drugs in the trial phase for COVID-19 treatment, the most promising and most effective drugs are discussed and summarized under the light of national guidelines and clinical evidences with all aspects of the literature.

Keywords: SARS-CoV-2, 2019-nCoV, COVID-19, drug treatment

ÖZ

Şiddetli akut solunum sendromu koronavirüs-2, ilk defa Çin'de tespit edilmiş, pandemiye ve binlerce insanın ölümüne yol açmış önemli bir enfeksiyon ajanıdır. Koronavirüs hastalığı-19 (COVİD-19) adı verilen hastalığın kliniği değişken olmakla birlikte neden olduğu sitokin fırtınası ve farklı mekanizmalar, ilerleyici ve ağır klinik tablolar sonucunda ölümle sonuçlanabilir. Virüsün izolasyonundan sonra özelliklerinin tanımlanması ve iyi bilinen bir virüs ailesine ait olması nedeniyle araştırmalar hız kazanmıştır. Hedefe yönelik uygun ilaçlar geliştirilmeye başlansa da ilk potansiyel aday ilaçlar, pandemiden daha önce benzeri endikasyonlar ile üretilmiş olan geniş spektrumlu antibiyotikler, antiviral ajanlar, anti-parazit ajanlar ve interferon gibi ilaçlardır. Profilaksi, destekleyici tedaviler, kritik hasta tedavisi ve aşı üzerine çalışmalar devam etmektedir. Kılavuzlar ışığında önerilen ilaçların kullanımı sonrasından ortaya çıkan yan etkiler ve etkinlik değerlendirmeleri sonucunda tedavi algoritmaları sıkça güncellenmiş ve bazı değişiklikler yapılmıştır. Özellikle bazı ilaç kombinasyonlarında QT süresinin uzama potansiyeli taşıması gibi yan etkilerin olması, ilaç-ilaç etkileşimleri, çocuk yaş grubu ya da gebelik döneminde kullanımıyla ilgili kısıtlamaların olması, özgün, kanıta dayalı ve güvenilir bir tedavinin olmasını kısıtlamaktadır. COVİD-19 tedavisi için deneme aşamasında birçok ilaç olmasına rağmen, en umut verici ve en etkili ilaçlar literatürdeki tüm yönleriyle ulusal kılavuzlar ve klinik kanıtlar ışığında tartışılmakta ve özetlenmektedir.

Anahtar Sözcükler: SARS-CoV-2, 2019-nCoV, COVİD-19, ilaç tedavisi

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Introduction

Coronavirus disease-2019 (COVID-19) is a pandemic and worldwide growing public health problem. There is currently no described specific established treatment for this disease. The beneficial and appropriate drug should be specific, effective in its highest level, proven with trials and suitable for pharmacological treatment. As the pandemic spreads rapidly and causes deaths, therapy is urgently becoming essential.

Some medicines which are utilized for other diseases, could potentially be beneficial for severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection without definitive evidences. At this point, the therapeutic approach should be based on infections coming from similar backgrounds with similar effects.

Structural parts of coronavirus (e.g. trimeric spike (S) protein, viral DNA) or some parts from life circle (e.g. proteases, hemagglutinin esterase, NTPase/helicase, and endosomal pH challenges) are important target points for developing drugs (1) (Figure 1).

The objective of this study is to examine drugs with a higher level of evidence, which are recommended or started to be used in COVID-19 treatment. Some of these medicines are known in literature with similar indications from the past. Under the light of current guidelines, these drugs are examined according to their recommendation level and effectiveness in use.

General Palliative/Supportive Treatment

Since there is no specifically defined treatment for SARS-CoV-2, the designated therapy should be appropriate for the clinical levels specific to the condition of the patients. Symptomatic treatment includes controlling high fever or pain with an antipyretic patch or antipyretic-analgesic drug treatment. The treatment regimen could be revised to comprehend a broad perspective. Supportive therapy is vital in these patients. General supportive treatment strategies for patients with mild symptoms are shown in Table 1 (2,3).

Medical Treatment with Drugs

There is a large number of drugs, which are still in the clinical trial phase to be used in the treatment of COVID-19 disease.

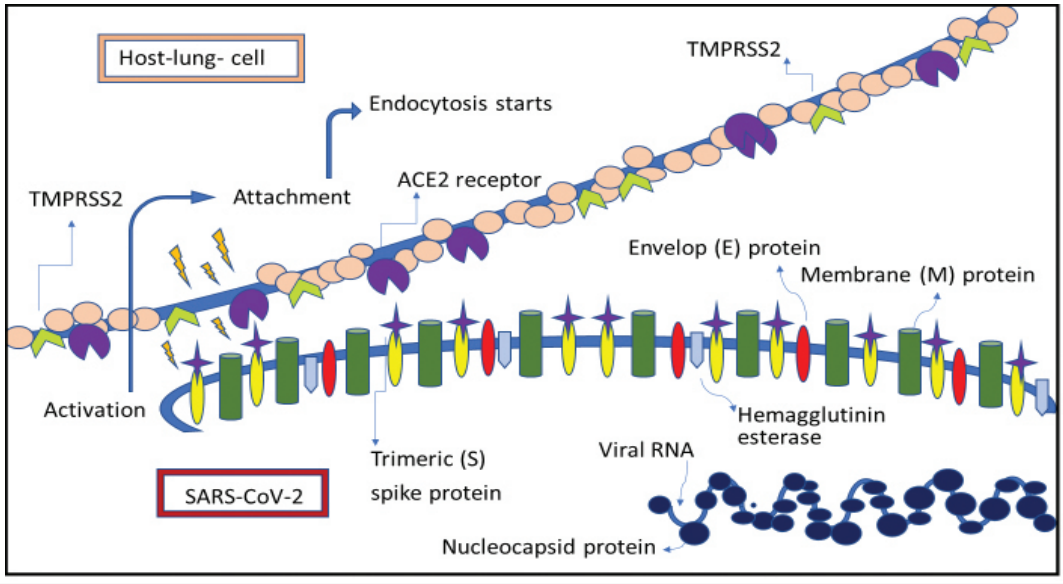

Figure 1. The structural parts of SARS-CoV-2
ACE-2: Angiotensin converting enzyme-2, TMPRSS2: Transmembrane serine protease 2, SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2

Table 1. Supportive treatment options other than specific medications (3)

General treatment strategies	Supportive care steps in critical illness
Bed resting	Prevent and treat complications, underlying diseases
Ensuring sufficient calory	Secondary bacterial or fungal infection treatment
Adequate water intake	Complete fluid resuscitation
Paracetamol	Lung protective ventilation strategy, O_2 support
İbuprofen	Prone position ventilation, lung recruitment
C vitamin	Vasoactive drugs
N-aceytil cysteine	ECMO

ECMO: Extracorporeal membrane oxygenation

Table 2. Classification of various drugs tried in COVID-19 (4,5)

Protease inhibitor	Interferon	Monoclonal antibody
-Lopinavir/ritonavir (/+ ribavirin)		-Tocilizumab
-Nelfinavir	-Interferon beta 1A	-Sarilumab (anti-IL6R)
-Camostat mesilate NI-03	-Interferon beta 1B	-SAB-301
-ASCO9 (/+ oseltamivir)	-PEG Interferon Lambda	-CDC2-C2
-ASCO9 (/+ ritonavir)	-Interferon Alpha 2A	-IFX-1
-Darunavir/cobicistat	-Interferon Alpha 2B	-M336
-Flavopiridol	-Interferon Alpha N3	-REGN3051 /+REGN3048
-Relacatib		
Anti-protozoal	RNA polymerase inhibitor	Immunemodulator
-Hydroxychloroquine	-Favipiravir	-Colchicine
-Chloroquine	-Favipiravir + interferon-α	-Hiltonol (poly-IC)
-Emetin		-Rintatolimod
Nucleoside analogue	Nucleoside analogue + reverse transcriptase inhibitor	Endonuclease inhibitor
-Remdesivir	-Emtricitabine + tenofovir	-Baloxavir morboxil
-Galidesivir		
Immunosuppressant	Fusion inhibitor	NMDA inhibitor
-Mycophenolate mofetil	-Arbidol (Umifenovir)	-Ifenprodil
Kinase inhibitor	Steroid	Neuraminidase inhibitör
-Baricitinib	-Hydrocortisone acetate	-Oseltamivir
Ribonucleoside analogue		
-Ribavirin		

Table 3. Various combination therapies in COVID-19 disease (4,5)

1. Drug		2. Drug		3. Drug
Corticosteroid	+	Rapamycine		
Hydroxychloroquine	+	Azithromycin		
Favipiravir	+	Oseltamivir		
Favipiravir	+	Tocilizumab		
Indinavir	+	Ritonavir		
Interferon beta	+	Mycophenolate mofetil		
Lopinavir/ritonavir	+	Ritonavir	+	Ribavirin
Lopinavir/ritonavir	+	İnterferon beta		
Ribavirin	+	İnterferon beta		
Ruxolitinib	+	Mesenchymal stem therapy		

Classification of drugs according to their effect mechanisms and possible combination therapies being utilized are given in Tables 2 and 3 (4,5).

Chloroquine

Chloroquine (CQ), a compound from class 4-aminoquinoline, has been used to treat malaria effectively as a cheap treatment based on its clinical safety.

CQ is also used for amebiasis, lupus erythematosus, Sjogren's syndrome and rheumatoid arthritis. Against rheumatoid arthritis, it acts by inhibiting lymphocyte proliferation, phospholipase A2, antigen presentation in dendritic cells, the release of enzymes from lysosomes, reactive oxygen species from macrophages, and production of interleukin (IL)-1 (6).

Lately, it was administered in CoV infections, SARS-CoV and Middle East respiratory syndrome-CoV (MERS-CoV). It has an effect on the immune system as an immunomodulating agent, inhibits cytokine production by suppressing T-cell activation, autophagy and lysosomal activity in antigen-presenting cells by preventing antigen processing and major histocompatibility complex class II-mediated autoantigen presentation to T-cells, and diminishes viral replication. There are ongoing and finished clinical trials on the use of CQ in COVID-19 disease because the efficacy and safety of CQ is still unsatisfactory (7).

Impaired terminal glycosylation of angiotensin-converting enzyme-2 (ACE-2) (the cellular receptor of SARS-CoV) may diminish the binding efficiency between ACE-2 on host cells and the SARS-CoV spike protein. Consequently, the binding of the virus to the receptors on the cells is interrupted and infection is averted. CQ also acts as a zinc ionophore, that permits of extracellular zinc insertion through the cell and inhibits viral RNA-dependent RNA polymerase (7).

Chloroquine vs Hydroxychloroquine?

CQ is used as phosphate and sulfate salt and it is stored in high concentrations in the liver, kidney, spleen, lungs and leukocytes. The half-life varies between 70 and 120 hours depending on the dose. It is slowly excreted from the body (8). The EC50 of CQ for SARS is 4.4 to 8.8 μM in *in vitro*, indicating that CQ could be more potent against COVID-19 than SARS (9).

In a study including 100 patients, CQ inhibited the exacerbation of pneumonia, promoted the conversion of the polymerase chain reaction test to a negative and shortened disease period however more detailed data were not reported (10).

Hydroxychloroquine, is an analogue of CQ, diverges from CQ with a single hydroxyl group attached to the end of the chain, by the way it provides low ocular toxicity. Nowadays, it is used more frequently due to the much lower side effects. Hydroxychloroquine has been shown more potent in vitro activity against SARS-CoV-2 than CQ. EC50 of Hydroxychloroquine is 0.72 μM for SARS-CoV-2 (8,11).

Although there are many data streams from all over the world, some of them indicate efficacy and acceptable safety for CQ in COVID pneumonia (5,10). In addition to that 23 trials are continuing, all in China. The trials are differed in study design, the severity of COVID-19 in the target group and application route and duration of the treatment (8).

A Chinese single center clinical trial (ChiCTR2000030417) which is not recruiting yet (n=30, 15/15); is comparing CQ phosphate as an aerosolized inhalation solution to water for atomized inhalation group (12).

In a clinical trial of 36 patients; a significant reduction of the viral carriage has been evidenced at day 6th after inclusion compared to controls with much lower average transfer duration compared to that of reported untreated patients in literature. This trial also shows decreased SARS-CoV-2 shedding with hydroxychloroquine treatment. The combination of hydroxychloroquine and azithromycin has a synergistic effect with a more efficient virus elimination, which, also provides viral carriage reduction. The clinical outcome is good (13).

In a limited study, 368 African American veteran males of different severity stages of COVID-19 disease were retrospectively evaluated. In all 3 groups; application of hydroxychloroquine and azithromycin, alone each or in combination, did not cause a significant decrease in the need for respiratory support and the number of deaths after ventilation. The increased risk of mortality was observed in the hydroxychloroquine group compared to the groups without hydroxychloroquine administration (14).

A study analysis with a multinational registry that was later retracted in June due to insufficient data advocated the restriction of hydroxychloroquine and CQ administration because of increased cardiovascular mortality detected in COVID-19 patients (15).

Dosing of Chloroquine

Dose arrangement is necessary between CQ phosphate and CQ base since 250 mg of the first one corresponds to 150 mg of the second. Based on pharmacokinetic modeling, a study recommends a dose for hydroxychloroquine 400 mg twice daily for the first day, then 200 mg twice daily for 4 days in the treatment of COVID-19, as it reached three times the potency of CQ phosphate when given 500 mg twice daily for 5 days (8).

Italian Society of Infectious and Tropical Disease, advises using CQ 500 mg twice a day or hydroxychloroquine 200 mg/day for 10 days and suggests adjusting treatment duration from 5 to 20 days, in line with the clinical severity (4).

For pediatric patients, it was also recommended in COVID-19 guideline of the Turkish Ministry of Health in different dosing regimens, 6.5 mg/kg/dose twice for the first day [maximum (max) 400 mg] and 3.25 mg/kg/dose twice for 2-5 days (16).

Side effects of CQ and some clinical conditions to be considered are shown in Tables 4 and 5, respectively (16,17).

Chloroquine in Pregnancy and Lactation

CQ has not been found to increase the risk of adverse fetal events or any harmful effects on the fetus when used in recommended doses for malarial prophylaxis, according to the American Centers for Disease Control and Prevention (CDC) guidelines. CQ and its metabolites pass the breast milk and placenta. It can be noticed in the cord blood and urine of the newborn infants (18).

There is not certain assigned pregnancy category by the Food and Drug Administration (FDA).

For children infected by SARS-CoV-2, there is no recommended dosage of CQ thus far. A dose of 50 mg/kg CQ refers to an acute poisoning and it can be fatal. In a report CQ serum concentration >25 μmol/L was considered as a predictor of lethality (18).

Mefloquine

As positive results have been published regarding the use of anti-malarial drugs in the treatment of COVID-19, recommendations began to emerge about another anti-malarial drug, mefloquine.

Mefloquine was found to have anti-viral activity against both MERS-CoV and SARS-CoV. The EC50 value of mefloquine is 7,416 for SARS-CoV and hydroxychloroquine sulfate is 7,966, CQ diphosphate is 6,538 (19).

Pangolin coronavirus GX-P2V is a workable model for SARS-CoV-2 research and the study has shown that mefloquine hydrochloride is a potential drug for treating SARS-CoV-2 infection (20).

Table 4. Side effects of chloroquine (17)

Unwanted/uncontrolled movements (including tongue and face twitching)
Deafness or tinnitus
Nausea, vomiting, diarrhea, abdominal cramps
Headache
Shortness of breath
Swelling legs/ankles, muscle weakness
Mental/mood changes (such as confusion, personality changes, unusual thoughts/behavior, depression, feeling being watched, hallucinating)
Signs of serious infection (such as high fever, severe chills, persistent sore throat)
Skin itchiness, skin color changes, hair loss, and skin rashes
Unpleasant metallic taste
Electrocardiographic changes*
Chloroquine retinopathy**
Pancytopenia, aplastic anemia, reversible agranulocytosis, low blood platelets, neutropenia, severe hypoglycaemia

*Electrocardiographic changes include conduction disturbances (bundle-branch block, atrioventricular block) or cardiomyopathy. Hypertrophy, restrictive physiology, and congestive heart failure often accompany the conditions which may be irreversible. Only two cases have been reported requiring heart transplantation, suggesting this particular risk is very low. Electron microscopies of cardiac biopsies show pathognomonic cytoplasmic inclusion bodies (67).
**Generally, chloroquine accumulates in retinal pigment cells and corneal epithelium. Accumulation in the cornea cause blurred vision; however, when the treatment is stopped, this effect will disappear but retinopathy tends to progress and can lead to permanent vision loss.

Table 5. Note some clinical situations before or simultaneously or afterwards proposing use of chloroquine (17)

Rule out the development of anemia, thrombocytopenia or leukopenia
Serum electrolyte disturbances
Hepatic and renal function dysfunction
Routine electrocardiography were recommended (rule out the development of QT interval prolongation or bradycardia)
Patient interviews to seek the appearance of visual and/or mental disturbance/deterioration
Avoid concurrent administration of other drugs known to prolong the QT interval (i.e. kinolones, macrolides, ondansetron) as well as various antiarrhythmic, antidepressant and antipsychotic drugs
Drug-drug interactions
Ask about G6PD deficiency; hepatic impairment, porphyria, seizure disorder*

*Use with caution in these patients; may exacerbate disease symptoms

Also, clinical trials are ongoing, about prophylaxis, efficacy, and safety of mefloquine. CDC suggests the anti-malarial drug mefloquine for pregnant women based on the recent FDA re-categorization of mefloquine from a pregnancy category C drug to category B.

Remdesivir

Remdesivir (GS-5734) is a mono phosphoramidate prodrug of an adenosine analog and it is manufactured by Gilead Sciences. It inhibits viral RNA polymerases activity of RNA virus families. It inhibits SARS-CoV and MERS-CoV replication in multiple *in vitro* systems, including primary human airway epithelial cell cultures at submicromolar IC_{50} values. This drug has also shown activity in a rhesus macque model of MERS-CoV infection. Remdesivir has been currently in clinical trials for the treatment of Ebola virus disease (11).

There has not been yet clearly stated any evidence of safety, optimal dosing, or effectivity for the treatment of COVID-19 disease, only *in vitro* activity against SARS-COV-2 has been shown (11). Under the light of the latest literature data, FDA has approved the use of this drug in COVID-19 on 1st of May, 2020 (emergency use authorization). The extensive guide published by the FDA provides detailed information about administration and side effects due to elevated transamine levels in the blood and infusion site reactions (21).

Immediately after remdesivir was approved by Japan on May 7, some trials like National Institute of Allergy and Infectious Diseases (NIAID) trial have resulted in some data as the drug helped patients heal faster than standard care, indicating it could become the first efficient therapy for the illness (22).

In a mouse model of SARS-CoV pathogenesis, the prophylactic and early therapeutic use of remdesivir markedly decreased lung viral load and improved clinical condition besides respiratory functions (11).

Airway resistance or accumulation of debris in the airway was markedly ($p<0.05$) elevated in vehicle-treated animals (mice) as compared to those treated with GS-5734. Prophylactic

administration of GS-5734 mitigated lung virus titers, improved lung function, and ameliorated symptoms of COVID-19. On the other hand, therapeutic post-exposure administration of GS-5734 appeased illness in this wide spectrum trial (23).

In an unpublished trial, which consists of 12 infected rhesus macaques with SARS-CoV-2, the treated group with remdesivir was significantly healthier than the untreated group and had a less viral load and less damage in lungs (24).

Fifty-three patients' data from multicenters were involved in a trial of compassionate use of remdesivir without any control groups. There were 2 deaths because of adverse effects resulting in liver and renal failure. Thirty-two patients (60%) were developed adverse events during the treatment period. The most common adverse events were increased hepatic enzymes, diarrhea, rash, renal impairment, and hypotension. Generally, adverse events were more severe in critical cases such as -multi-organ-dysfunction syndrome, septic shock, and hypotension (25).

There is an evidence of *in vivo* administration of remdesivir for prophylactic and therapeutic use against MERS-CoV infections in mice. Remdesivir improves pulmonary function and severe lung pathology, as well as reduces viral titers in the lung (26). Further, experiments in mice using remdesivir showed higher efficacy than lopinavir/ritonavir + interferon (IFN)-β (27).

Moderate or severe ill COVID-19 cases with respiratory problems who received remdesivir, recovered quickly than similar patients who received placebo, according to a preliminary data analysis from a randomized, controlled trial including 1063 hospitalized patients (28).

In contrast, in a double-blind randomized trial including 237 Chinese patients with severe COVID-19 (hypoxia and radiographically confirmed pneumonia), duration to clinical healing was not statistically different with remdesivir compared to placebo for 10 days (29).

NIAID investigates therapeutics, as there is not a clear dose regimen schema for adults and adolescents with acute Ebola virus disease, it suggests as following; single remdesivir 200 mg IV loading dose on the first day of treatment followed by 9th to 13th day (if virus has still been detectable in plasma) once daily 100 mg IV maintenance dose. It has an intracellular half-life of longer than 35 hours.

For pediatric patients with body weight <40 kg, a body weight-based dosing regimen of one loading dose of remdesivir 5 mg/kg IV (max 200 mg) on day 1, followed by 2.5 mg/kg/dose IV once daily (max 100 mg) was used in 41 pediatric patients involving 2 neonates who were in a phase 3 Ebola trial (30).

Ribavirin

Ribavirin, a guanine analogue, which inhibits viral RNA-dependent RNA polymerase in other coronaviruses, is also a candidate for COVID-19 treatment. Nowadays, only *in vitro* data is available regarding the activity of ribavirin on SARS-CoV-2. Ribavirin seems to be less potent *in vitro* than CQ and remdesivir (11). In a systematic review of ribavirin treatment in patients infected with SARS-CoV, 26 trials were inconclusive, and 4 showed potential harm as in Canada 2003. Additionally, it decreased the amount of hemoglobin as a side effect in cases needing respiratory support (27,31).

An open-label study resulted in 2004, compared a historical control group of 111 cases who were administered only ribavirin with 41 cases who were received a combination of lopinavir/ritonavir (400 mg/100 mg) and ribavirin. Ribavirin alone had no significant beneficial effect in reducing adverse clinical outcomes such as death and reducing the viral load of SARS-CoV (32).

Interferons

Interferons have antiviral properties and they have been evaluated as a potential treatment for COVID-19 disease but with lack of data in this regard. Some clinical trials have not been yet published or concluded, support of using interferons, alone or in a combination.

A clinical trial of 350 critically ill patients with MERS-CoV was evaluated. Mortality rates were higher in patients who received ribavirin and interferon than compared to the group without any medication (33). In 301 patients with ARDS, intravenous IFN-β1-α administration had almost same ventilation time and mortality rate compared to placebo (34). IFN-β is better tolerated in terms of its side effects than IFN-α.

Lopinavir/Ritonavir

Lopinavir, a repurposed drug for the treatment of MERS-CoV and SARS-CoV, is an antiretroviral medicine primarily used for human immunodeficiency virus (HIV) infection (27,32). Lopinavir is combined with ritonavir, which inhibits the cytochrome p450, by the way plasma half lifetime of lopinavir is extended.

As it has been used for the treatment of HIV-positive pregnants, there is a wide experience for good safety. Although lopinavir is found in the breast milk at a much lower amount, according to World Health Organization (WHO) guidelines, several safer treatment options are recommended to treat HIV-infected mothers (35). It was also recommended by Turkish Ministry of Health, COVID-19 guidelines for use in pediatric patients (16).

According to a clinical trial of the combination of lopinavir/ritonavir with ribavirin or IFN-α, viral load was reduced, and survival improved among SARS and MERS patients (32,36).

Retrospective comparative analysis in MERS, lopinavir/ritonavir ensured ARDS or death reduction from 28% to 2.4% (27).

A randomized clinical trial was compared lopinavir-ritonavir therapy and standard-care therapy in hospitalized SARS-CoV-2 infected patients. There was no clinical improvement or difference in mortality at 28 days or detectable viral RNA load at various time points. Nevertheless, gastrointestinal adverse events were more common. Although skin eruptions, QT prolongation, pancreatitis, drug interactions due to CYP3A inhibition was observed in the lopinavir-ritonavir group, serious adverse

events/complications such as ARDS or acute kidney injury and pneumothorax was more common in the standard-care group (37).

Favipiravir

Favipiravir (T705) is a purine nucleic acid analog of pyrazine carboxamide derivative (6-fluoro - 3-hydroxy - 2-pyrazinecarboxamide), which is an antiviral drug developed for the treatment of influenza and it was used during the Ebola virus outbreak. It increased the survival rate and reduced viral load in Ebola virus-infected patients. Nowadays, it is being evaluated for the safety and efficacy in patients with COVID-19 (38). So far registered clinical trials are expected to be beneficial. Clinical trials are ongoing about different dosing regimens of favipiravir alone or combined with tocilizumab, CQ, IFN-α and, other antiviral treatments for the use of COVID-19 disease. In a clinical trial, no evidence of difference in the improvement of patients' condition between favipiravir and arbidol treatment has been shown (39).

For dosing there is a preliminary *in vitro* and preclinical data from China that the regiment of 3,200 mg/day loading dose on day 1, followed by 1,200 mg maintenance dose on day 2 to day 14 is effective or, a loading dose is recommended (2400 mg to 3000 mg every 12 hours, 2 doses/day) and followed by a maintenance dose (1200 mg to 1800 mg every 12 hours). The half-life of favipiravir is approximately 5 hours (40).

Corticosteroids

ARDS is the major lethal clinical picture due to SARS-CoV-2 infection**.** Corticosteroids can reduce the host inflammatory responses in the lungs with potent anti-inflammatory effects and a potential therapeutic role in suppressing cytokine-associated lung damage with acute lung injury.

During community-acquired pneumonia, the effect of corticosteroid treatment was investigated through the systematic review and meta-analysis of randomized trials. Reduced parameters were found to be mortality (3%) and mechanical ventilation support (5%), and length of hospital stay (about 1 day) (41).

Generally, there is no certain data came up to expect that COVID-19 disease will benefit from corticosteroids. On the other hand, their harmfulness must not be ignored. Some authors conclude that corticosteroid treatment should not be administered for COVID-19-related lung injury treatment or shock without conducting clinical trials. WHO guideline also recommends not to use systemic corticosteroids for the treatment of any viral pneumonia except during clinical trials (42).

The Chinese Thoracic Society has published an expert consensus declaration involved fundamental principles when utilizing corticosteroids in SARS-CoV-2 pneumonia: the benefits and harms should be weighed prior to administering corticosteroids, corticosteroids should be used carefully in advanced stages of illness with SARS-CoV-2 pneumonia, for patients with hypoxemia due to COVID-19 or who regularly use corticosteroids for chronic diseases for further use of corticosteroids should be cautious, and the dose regimen should be low-to-moderate (≤0.5-1 mg/kg/day methylprednisolone or equivalent) and the therapy period should be less than almost 7 days (43).

Infectious Diseases Society of America Guidelines recommends corticosteroid administration in the patients who hospitalized with ARDS due to COVID-19, not all the hospitalized COVID pneumonia (44).

In contrast, different meta-analysis results of 528 patients showed that prolonged corticosteroid therapy is more effective in severe community-acquired pneumonia to reduce the mortality and risk of ARDS (45). In a systematic review consisting steroid use in 29 COVID-19 patients, 25 were inconclusive and 4 were categorized as causing possible harm (31).

In addition, increased mortality was observed due to the early administration of corticosteroids in 241 patients with Influenza-associated ARDS (46).

Corticosteroid administration should be evaluated on a case-by-case basis, based on the patient's disease status and severity, indication and underlying medical condition. More clinical research and data are needed.

Vitamin C

Beyond antiviral and antioxidant properties, vitamin C has effects on immune system response. It avoids epithelial water-channel damage due to the limitation of active neutrophil migration to alveolar medium and support for the development of more channels. It is caused by the enhancement of alveolar fluid clearance. Also, it abates lipopolysaccharide-induced acute lung injury.

Short-term high-dose vitamin C in selected patients may improve hemodynamic parameters, decrease fluid resuscitation requirements, reduce the incidence of perioperative atrial fibrillation, improve pain, and potentially reduce sepsis-associated mortality. High-dose intravenous vitamin C has also been successfully applied to 50 moderate to severe COVID-19 cases. The oxygenation index was healed in real time and all the cases finally cured (47).

On the other hand, the concern that may occur with high-dose vitamin C therapy is osmotic cell death of immune cells, but not apoptosis, which could generate a local inflammation in alveolar space. Accordingly, intravenous glucocorticoid treatment must be performed to limit possible inflammatory damage of high-dose vitamin C therapy (48).

Also, analysis of the CITRIS-ALI study has shown the efficacy of high dose vitamin C (50 mg/kg /6 hrs.) in preventing mortality from acute lung injuries as compared to placebo. This should be considered for evaluation in COVID-19 treatment (49).

Teicoplanin

Teicoplanin is a glycopeptide antibiotic used in the treatment of gram-positive bacterial infections, especially staphylococcal infections. Teicoplanin acts on an early stage of the coronaviruses

and also SARS-COV-2 life cycle by inhibiting the low-pH cleavage of the viral spike protein by cathepsin-L in the late endosomes, thus avoiding the dissemination of genomic viral RNA and proceeding the virus replication cycle. Recently an experiment showed that this activity was conserved against SARS-CoV-2 (the target sequence that serves as the cleavage site for cathepsin L is conserved among SARS-CoV spike proteins) (50). Once these preliminary results have been confirmed in a randomized clinical trial, teicoplanin will be useful for the treatment of COVID-19 (51).

Tocilizumab

Tocilizumab, an IL-6 receptor-inhibiting monoclonal antibody, was first approved by FDA for the treatment of rheumatoid arthritis, juvenile idiopathic arthritis, giant cell arteritis, and chimeric antigen receptor T-cell-induced severe or life-threatening cytokine release syndrome. The beneficial effect is against the amplified pathological hyperactivity of the immune response observed in COVID-19.

According to a study of 21 patients with severe or critical COVID-19, partial improvement in clinical condition and CT scan image was shown after administration of 400 mg tocilizumab mostly once, in addition to their routine care (52). There are 14 ongoing clinical trials about using tocilizumab for COVID-19 in different dose regimens, alone or in combination, administered intravenously within no less than 60 minutes (53).

The Chinese National Health Commission guidelines suggested using tocilizumab in the management steps of severe COVID-19 with widespread bilateral lung disease and elevated IL-6 (5). The SIMIT Lombardy section guidelines recommended tocilizumab in critically ill patients with ARDS. Side effects can be allergic reactions, fever, chills, increased risk of serious infections, or bleeding, gastritis, liver damage and hepatic failure, leukopenia, neutropenia, thrombocytopenia, muscle weakness, dyspnea, hypertension, etc (4).

Monoclonal antibodies are actively transported across the placenta during the last trimester (pregnancy category C). It is a large protein molecule, the amount in breast milk is likely to be very low and absorption is unlikely because it is probably destroyed in the infant's gastrointestinal tract (52).

Emetine

Emetine is a protein synthesis inhibitor that was used as anti-protozoan approved for the treatment of ameobiasis, it also inhibits malaria by binding to the ribosomal E site of *Plasmodium falciparum.* However, it has been used less frequently over the years because it has a significant cardiotoxic effect.

Emetine has antiviral efficacy against a wide range of RNA and DNA viruses and inhibits human CoV *in vitro* with EC_{50} reported at low micromolar. Also, emetine was observed at nearly 0.5 μM efficiently interrupting SARS-CoV-2 virus replication in Vero E6 cells (54).

Ivermectin

It is confirmed by the FDA as a broad-spectrum anti-parasitic effect for the treatment of cutaneous larva migrants. In recent studies, ivermectin has been shown to have an *in vitro* effect on SARS-CoV-2 caused by reduction in viral RNA without causing any toxicity raised from the nuclear importin-α/β-mediated transport inhibitory activity. Clinical trials are underway that explore the efficacy of ivermectin alone by real-life experience or combination with hydroxychloroquin and azithromycine, nitazoxanide. Due to their consequential and synergistic manner, ivermectin is recommended as a proposition to use in combination with hydroxychloroquine with no serious drug-related adverse events (55).

In a clinical study 1400 hospitalized SARS-CoV-2 infected patients were treated with 150 mcg/kg of ivermectin once while the second group (control group) adhered to the treatment protocol (not included ivermectin). It was found that there was a decrease in hospitalization period and mortality in the ivermectin group (56).

Oseltamivir

Oseltamivir is a neuraminidase inhibitor that has a proven beneficial effect on prophylaxis and treatment of influenza. It has taken its place among the treatment alternatives of COVID-19 due to its low side effects such as nausea and vomiting. There isn't any identified enzyme or mechanism indicating the effectivity of oseltamivir on coronavirus. Thus, its mechanism of action is not clear. Even so, it has participated in combinations of antiviral therapy in China (57). Trials evaluating only combination regimens are underway.

Intravenous Immunoglobulin and other Immunomodulator Agents

Intravenous immunoglobulin (IVIG) has many indications as a part of combination therapies in treatment of several diseases including adjuvant therapy in a more pathogen-focused (hyperimmune) form.

Convalescent (immune) plasma is a kind of plasma obtained from the patients recently recovered SARS-CoV-2 infection. It has been accepted as passive antibody therapy in the other CoV infections as well. The issues are regarding its application quantity to appropriate patients. It is recommended to be used in the last-line treatment group, especially in critically ill patients (58). Nevertheless, there are few case reports regarding administration of IVIG for COVID-19 treatment (58). The results of 7 trials of convalescent plasma or IVIG on the SARS-infected patient were inconclusive (31).

During the SARS outbreak, approximately 30% of critically ill patients developed venous thromboembolism despite the prophylactic use of low-molecular weight heparin. It was supposed to be developed due to the IVIG-induced increase of viscosity in hypercoagulable states of SARS patients (59).

Although there are different views about the use of IVIG, a study was conducted in 58 SARS-CoV-2 infected patients. IVIG

administration within 48 hours of admission of the patients to the intensive care unit, has been shown to be effective in reducing the use of mechanical ventilation, hospital length of stay and mortality of patients with severe COVID-19 pneumonia (60).

Immunomodulatory agents can be necessary before multi-organ dysfunction to attenuate systemic inflammation. Corticosteroids may use in compatibly with cytokine inhibitors such as tocilizumab or anakinra (IL-1 receptor antagonist). Generally, recovery from this advanced severe stage of illness is rare, and immediate recognition of condition and administration of such therapy may be the most vital point (61).

Hemophagocytic lymphohistiocytosis (HLH), is a severe and rare condition which can be develop in no more than 0.25-1% of COVID-19 patients during cytokine storm syndrome. Corticosteroids, IVIG, tocilizumab, anakinra, JAK inhibitors and even chemotherapeutics can be used for treatment of HLH. IVIG treatment can be administered for 2 days at the dose of 2 g/kg/day with immunoglobulin level monitoring (should not be used in IgA deficiency) (16).

COVID-19 Treatment Guidelines Panel suggests not to use the following drugs for the treatment of COVID-19: The combination of hydroxychloroquine plus azithromycin because of the potential for toxicities and lopinavir/ritonavir or other HIV protease inhibitors because of unfavorable pharmacodynamics and negative clinical trial data. Except in the context of a clinical trial, the Panel advised against the use of other immunomodulators, such as IFNs, because of lack of efficacy in the treatment of SARS and MERS and their toxicity and Janus kinase inhibitors (e.g., baricitinib), due to their broad immunosuppressive effect (62).

Umbilical Cord Mesenchymal Stem Cell (UC-MSC) Transplantation

According to the clinical data, UC-MSCs and MCS have anti-inflammatory, immunomodulatory and antimicrobial actions, thereby, it can heal damaged tissues. The target tissue for stem cells is where they are caught by the lungs, as this is a favorable condition for COVID-19 treatment.

The clinical condition of elderly patients or patients with secondary comorbidities may progress worse and may not response to conventional treatment. For critically ill patients, infected with SARS-CoV-2, MSC therapy is the last resort and it can save lives. Unfortunately, even if MCS treatment has been previously attempted in different diseases, it is a new and inexperienced treatment method for COVID-19 (63).

In a clinical study presenting positive responses of seven COVID-19 pneumonia patients to MSC, genetic examination showed that MSCs are negative for ACE2 and the cellular protease TMPRSS2, which are known to be exempt from COVID-19 infection. Bone marrow, lymph nodes, thymus, and the spleen, immune cells, such as T and B lymphocytes, and macrophages were consistently negative for ACE2, as well. Therefore, intravenous transplantation of MSCs is likely to be a safe and effective option for the treatment of patients with COVID-19 pneumonia at the critically severe stage (63,64).

Vaccine Developments

It is necessary to overcome various difficulties to succeed in vaccine production. Many vaccine studies are carried out worldwide in accordance with phase 1 through phase 4 trial steps (65).

Moderna, Inc. is manufacturing therapeutics and vaccines for infectious diseases, immuno-oncology, rare diseases and cardiovascular diseases with collaborators as the Vaccine Research Center at the NIAID. mRNA-1273 is an mRNA vaccine, developed by Moderna, against SARS-CoV-2 encoding for a prefusion stabilized form of the Spike (S) protein. mRNA-1273 inhibits viral replication in the lungs of mice in the responding stage to SARS-CoV-2 infection. It is finalizing the protocol for a Phase 3 trial, phase IV "post-approval" studies will probably start in July 2020 (65,66).

Conclusion

Currently, no specific treatment has been approved by the FDA in the treatment or prophylaxis of COVID-19 disease, except for Remdesivir. Despite the claim in medical literature and some clinical studies regarding the achievement of cure in COVID-19 patients using various therapeutics, appropriate clinical research results are needed to identify optimal treatments for COVID-19.

For the current clinical management, supportive care is recommended, including infection prevention and control measures and respiratory support when indicated. As with general patient management, the healthcare provider makes decisions based on the patient's condition and characteristics.

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References

1. Prajapat M, Sarma P, Shekhar N, Avti P, Sinha S, Kaur H. Drug targets for corona virus: A systematic review. Indian J Pharmacol 2020:52:56-65.
2. Özdemir Ö, Pala A. Çocuklarda COVID-19 Enfeksiyonunun Tanısı, Tedavisi ve Korunma Yolları. J Biotechinol and Strategic Health Res 2020;4:21-14.
3. Abd El-Aziz TM, Stockand JD. Recent progress and challenges in drug development against COVID-19 coronavirus (SARS-CoV-2) - an update on the status. Infect Genet Evol 2020;83:104327.

4. Lombardy Section Italian Society I, Tropical D. Vademecum for the treatment of people with COVID-19. Edition 2.0 Infez Med 2020;28:2:143-52.
5. National Health Commission of the People's Republic of China. Guidelines for the Prevention, Diagnosis, and Treatment of Novel Coronavirus-induced Pneumonia. 7th ed; 2020 Available from: http://www.gov.cn/zhengce/zhengceku/2020-03/04/5486705/files/ae61004f930d47598711a0d4cbf874a9.pdf
6. The American Society of Health-System Pharmacists. Aralen Phosphate. 2015 Access date: 5 may 2020 Available from: https://www.ashp.org/?loginreturnUrl=SSOCheckOnly
7. Zhou D, Dai SM, Tong Q. COVID-19: A recommendation to examine the effect of hydroxychloroquine in preventing infection and progression. J Antimicrob Chemother 2020;75:1667-70.
8. Cortegiania A, Ingoglia G, Ippolitoa M, Giarratanoa A, Einav S. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. J Crit Care 2020;57:279-83.
9. J-Marc C, Jean R, Lagierab C, Brouquiab P, Raoul D. Chloroquine and hydroxychloroquine as available weapons to fight COVID-19. Int J Antimicrob Agents 2020;55:105932.
10. Gao J, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends 2020;14:1:72-3.
11. Wang M, Cao R, Zhang L, Yang X, Liu L, Xu M, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 2020;30:3:269-71.
12. Efficacy and safety of chloroquine phosphate inhalation combined with standard therapy in the treatment of novel coronavirus pneumonia (COVID-19) Last Accessed Date: 17.03.2020. Available from: https://apps.who.int/trialsearch/Trial2.aspx?TrialID=ChiCTR2000030417
13. Gautret P, Lagier JC, Parola P, Meddeb L, Mailhe M, Doudier B, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents 2020; 56(1):105949.
14. Magagnoli J, Narendran S, Pereira F, Cummings T, Hardin JW, Sutton SS, et al. Outcomes of hydroxychloroquine usage in United States veterans hospitalized with Covid-19. Medrxiv 2020;04:20065920.
15. Mehra MR, Desai SS, Frank Ruschitzka F, Patel AN. Hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis. Lancet 2020;395:1820.
16. COVID-19 (SARS-CoV2 Infection) Directory (Science Committee Work). T.R. Ministry of Health, 2 April 2020
17. WebMD. Chloroquine Side Effects: Common, Severe, Long Term. Last Accessed Date: 22.03.2020. Available from: www.webmd.com. https://www.webmd.com/drugs/2/drug-8633/chloroquine-oral/details
18. Centers for Disease Control and Prevention. CDC Health Information for International Travel 2018. New York: Oxford University Press; 2018.
19. Dyall J, Coleman CM, Hart BJ, Venkataraman T, Holbrook MR, Kindrachuk J, et al. Repurposing of clinically developed drugs for treatment of Middle East respiratory syndrome coronavirus infection. Antimicrob Agents Chemother 2014;58:4885-93.
20. Fan HH, Wang LQ, Liu WL, Ann XP, Liu ZD. Repurposing of clinically approved drugs for treatment of coronavirus disease 2019 in a 2019-novel coronavirus-related coronavirus model. Chin Med J (Engl) 2020;133:1051-6.
21. Food and Drug Administration. Fact sheet for health care providers: Emergency use authorization of remdesivir. Last AccessHYPERLINK "https://www.fda.gov/media/137566/download%20access%20date%20May%202020"
22. Japan Approves Gilead's Remdesivir to Treat Covid-19, NHK Says. Bloomberg Last Accessed Date: 07.03.2020. Available from: https://www.bloomberg.com/news/articles/2020-05-07/japan-set-to-approve-remdesivir-for-coronavirus-on-thursday
23. Sheahan TP, Sims AC, Graham RL, Menachery VD, Gralinski LE, Case JB, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med 2017;9:eaal3653.
24. Williamson BN, Feldmann F, Schwarz B, Meade-White K, Porter DP, Schulz J, et al. Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2. bioRxiv [Preprint]. 2020.04.15.043166.
25. Grein J, Ohmagari N, Shin D, Diaz G, Asperges E, Castagna A. Compassionate Use of Remdesivir for Patients with Severe Covid-19. N Engl J Med 2020;382:2327-36.
26. Sheahan TP, Sims AC, Leist SR, Schafer A, Won J, Brown AJ, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun 2020;11:222.
27. WHO R&D Blueprint Informal consultation on prioritization of candidate therapeutic agents for use in novel coronavirus 2019 infection Geneva, Switzerland. Last Accessed Date: 24.01.2020. Available from: https://apps.who.int/iris/bitstream/handle/10665/330680/WHO-HEO-RDBlueprint%28nCoV%29-2020.1-eng.pdf
28. NIH Clinical Trial Shows Remdesivir Accelerates Recovery from Advanced COVID-19. (cited 2020 April, 29) Available from: https://www.niaid.nih.gov/news-events/nih-clinical-trial-shows-remdesivir-accelerates-recovery-advanced-covid-19
29. Wang Y, Zhang D, Du G, Du R, Zhao J, Jin Y, et al. Remdesivir in adults with severe COVID-19: A randomised, double-blind, placebo-controlled, multicentre trial. Lancet 2020; 395: 1569-78.
30. National Institute of Allergy and Infectious Diseases (NIAID). Investigational Therapeutics for the Treatment of People With Ebola Virus Disease. ClinicalTrials.gov Identifier: NCT03719586. March 2020. Available from: https://clinicaltrials.gov/ct2/show/NCT03719586? term=randomized+ebola&draw=2.
31. Stockman LJ, Bellamy R, Garner P. SARS: systematic review of treatment effects. PLoS Med 2006;3:9:e343.
32. Chu CM, Cheng VC, Hung IF, Wong M, Chan K, Kao R, et al. Role of lopinavir/ritonavir in the treatment of SARS: Initial virological and clinical findings. Thorax 2004;59:252-6.
33. Arabi YM, Shalhoub S, Mandourah Y. Ribavirin and interferon therapy for critically ill patients with Middle East respiratory syndrome: a multicenter observational study. Clin Infect Dis 2020;70:1837-44.
34. Ranieri VM, Pettila V, Karvonen MK, Jalkanen J, Nightingale P, Brealey D, et al. Effect of intravenous interferon beta-1a on death and

days free from mechanical ventilation among patients with moderate to severe acute respiratory distress syndrome: a randomized clinical trial. JAMA. 2020 Feb 17. doi: 10.1001/jama.2019.22525. Online ahead of print. https://www.ncbi.nlm.nih.gov/pubmed/32065831.

35. Recommendations for the Use of Antiretroviral Drugs in Pregnant Women with HIV Infection and Interventions to Reduce Perinatal HIV Transmission in the United States. (Cited 2020 April) Available from: https://aidsinfo.nih.gov/guidelines/html/3/perinatal/0
36. Kim UJ, Won EJ, Kee SJ, Jung SI, Jang HC. Combination therapy with lopinavir/ritonavir, ribavirin and interferon-α for Middle East respiratory syndrome. Antivir Ther 2016;21:455-9.
37. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, Ruanet L, et al. A Trial of Lopinavir-Ritonavir in Adults Hospitalized with Severe Covid-19. N Engl J Med 2020;382:1787-99.
38. Bai CQ, Mu JS, Kargbo D, Song YB,Niu WK, Nie WM, et al. Clinical and virological characteristics of Ebola virus disease patients treated with favipiravir (T-705)-Sierra Leone, 2014. Clin Infect Dis 2016:63;1288-94
39. Chen C, Huang J, Cheng Z, Wu J, Chen S, Zhang Y, et al. Favipiravir versus Arbidol for COVID-19: a randomized clinical trial. medRxiv 2020.03.17.20037432.
40. Cai Q, Yang M, Liu D, Chen J, Shu D, Xia J, et al. Experimental treatment with Favipiravir for COVID-19: An Open-Label Control Study. Engineering https://doi.org/10.1016/j.eng.2020.03.007. (e-pub ahead of print].
41. Siemieniuk RAC, Meade MO, Alonso-Coello P, Briel M, Evaniew N, Prasad M, et al. Corticosteroid Therapy for Patients Hospitalized With Community-Acquired Pneumonia: A Systematic Review and Meta-analysis. Ann Intern Med 2015;163:519-28.
42. Russell CD, Millar JE, Baillie JK. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. Lancet 2020;395:10223:473-5.
43. Zhao JP, Hu Y, Du RH, Chen ZS, Jin Y, Zhou M, et al. Expert consensus on the use of corticosteroid in patients with 2019-nCoV pneumonia (in Chinese). Zhonghua Jie He He Hu Xi Za Zhi 2020;43:E007.
44. Sun F, Kou H, Wang S, Yun L, Zhao H, Li W, et al. Medication Patterns and Disease Progression Among 165 Patients with Coronavirus Disease 2019 (COVID-19) in Wuhan, China: A Single-Centered, Retrospective, Observational Study. SSRN Electronic Journal. 10.2139/ssrn.3551323.
45. Bi J, Yang J, Wang Y, Yao C, Mei J, Liu Y, et al. Efficacy and safety of adjunctive corticosteroids therapy for severe community-acquired pneumonia in adults: an updated systematic review and meta-analysis. PLoS One 2016;15:e0165942.
46. Tsai MJ, Yang KY, Chan MC, Kao KC, Wang HC, Perng WC, et al. Impact of corticosteroid treatment on clinical outcomes of influenza-associated ARDS: A nationwide multicenter study. Ann Intensive Care 2020:27;10:26.
47. Shanghai Expert Consensus on Covid-19 Treatment. Last Accessed Date: 23.03.2020. Available from: http://www.drwlc.com/blog/2020/03/21/shanghai-expert-consensus-on-covid-19-treatment/
48. High-dose intravenous vitamin C treatment for COVID-19 (a mechanistic approach) Erol Project Development House for the disorders of energy metabolism Silivri-Istanbul, Turkey DO-10.13140/RG.2.2.28639.20646
49. Fowler AA, Truwit JD, Hite RD, Morris PE, DeWilde C, Priday A, et al. Effect of Vitamin C Infusion on Organ Failure and Biomarkers of Inflammation and Vascular Injury in Patients With Sepsis and Severe Acute Respiratory Failure: The CITRIS-ALI Randomized Clinical Trial. JAMA 2019:1;322:1261-70.
50. Zhang J, Ma X, Yu F, Liu J, Zou F, Pan T, et al. Teicoplanin potently blocks the cell entry of 2019-nCoV. bioRxiv 2020.02.05.935387
51. Baron SA, Devaux C, Colson P, Raoult D, Rolain JM. Teicoplanin: an alternative drug for the treatment of COVID-19? Int J Antimicrob Agents 2020;55:105944.
52. Xu X, Han M, Li T, Sun W, Wang D, Fu B, et al. Effective treatment of severe COVID-19 patients with tocilizumab. Proc Natl Acad Sci USA 2020;117:10970-5
53. NIH. Tocilizumab. Clinical Trials. Last Accessed Date: 28.04.2020. Available from: https://clinicaltrials.gov/ct2/results?cond=covid-19+tocilizumab&term=&cntry=&state=&city=&dist=&Search=Search
54. Choy KT, Wong A, Kaewpreedee P, Sia SF, Chen D, Yan Hui KP, et al. Remdesivir, lopinavir, emetine, and homoharringtonine inhibit SARS-CoV-2 replication in vitro. Antiviral Res 2020;178:104786.
55. Patrì A. Fabbrocini G. Hydroxychloroquine and ivermectin: a synergistic combination for COVID-19 chemoprophylaxis and/or treatment? J Am Acad Dermatol 2020;82:e221.
56. Patel AN, Desai AS, Grainger DW, Mehra MR. Usefulness of Ivermectin in COVID-19 Illness. Last Accessed Date: 19.04.2020. Available from: https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3580524 under review by SSRN.
57. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020;395:10223:507-13.
58. Özdemir Ö, Melek Arsoy HE. Convalescent (immune) plasma therapy with all aspects: yesterday, today and COVID-19. Erciyes Med J 2020;42:252-9.
59. Dalakas MC, Clark WM. Strokes, thromboembolic events, and IVIg: Rare incidents blemish an excellent safety record. Neur 2003;60:1736-7.
60. Xie Y, Cao S, Li Q, Chen E, Dong H, Zhang W, et al. Effect of regular intravenous immunoglobulin therapy on prognosis of severe pneumonia in patients with COVID-19 J Infect 2020;81:318-56.
61. Siddiqi HK, Mehra MR. COVID-19 Illness in Native and Immunosuppressed States: A Clinical-Therapeutic Staging Proposal. J Heart Lung Transplant 2020;39:405-7.
62. NIH. Potential Antiviral Drugs Under Evaluation for the Treatment of COVID-19. Last Accessed Date: 12.05.2020. Available from: https://www.covid19treatmentguidelines.nih.gov/therapeutic-options-under-investigation/
63. Leng Z, Zhu R, Hou W. Transplantation of ACE2 Mesenchymal stem cells improves the outcomes of patients with COVID-19 pneumonia. Aging Dis 2020;11:216-28.

64. Hamming I, Timens W, Bulthuis MLC, Lely AT, Navis GJ, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathology 2004:203:631-7.

65. Moderna . Moderna Announces Positive Interim Phase 1 Data for its mRNA Vaccine (mRNA-1273) Against Novel Coronavirus. Last Accessed Date: 18.03.2020. Available from: https://investors.modernatx.com/node/8986/pdf

66. Amanat F, Krammer F. SARS-CoV-2 Vaccines: Status Report. Immunity 2020:52;4;583-9

67. Tönnesmann E, Kandolf R, Lewalter T. Chloroquine cardiomyopathy - A review of the literature. Immunopharm Immunotoxicol. 2013:35:434-42.

Review

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Potential Treatment Approaches to SARS-CoV-2 and Evaluation of Drug Carrier Systems in Treatment

SARS-CoV-2'ye Yönelik Potansiyel Tedavi Yaklaşımları ve İlaç Taşıyıcı Sistemlerin Tedavide Değerlendirilmesi

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ABSTRACT

The severe acute respiratory syndrome-coronaviruse-2 (SARS-CoV-2) genome is packaged in a helical nucleocapsid surrounded by a lipid bilayer. The virus envelope contains at least three viral proteins called spike protein (S), membrane protein (M) and envelope protein (E). While M and E form the structure of the virus, S protein is the leading agent of the entry of viruses into the host. Angiotensin converting enzyme-2 (ACE-2) has been identified as a functional receptor for coronaviruses, including SARS-CoV and SARS-CoV-2. Viral fusion is the main step in the onset of SARS-CoV-2 infection. It is thought that drugs that prevent spike protein and ACE-2 fusion, drugs acting on the renin-angiotensin-aldesterone system, and a high dose ACE-2 can act on this fusion mechanism and take part in COVID-19 treatment. In this context, especially nano-sized liposomal carriers attract attention due to their biocompatibility and cell-like structures in the treatment of infectious diseases. There are studies in which liposomes are also used as a secondary therapeutic to support traditional anti-infective drugs. In this review, therapeutic approaches that may reduce and treat the severity of the disease by preventing ACE-2 mediated entry of viruses are discussed.

Keywords: COVID-19, SARS-CoV-2, liposomes, COVID-19 treatment

ÖZ

Şiddetli akut solunum yolu enfeksiyonu sendromu-koronavirüs-2 (SARS-CoV-2) genomu, lipit çift tabakası ile çevrelenen sarmal bir nükleokapsid içine paketlenmiştir. Virüs zarfı, spike proteini (S), zar proteini (M) ve zarf proteini (E) olarak isimlendirilen en az üç viral protein içermektedir. M ve E virüsün yapısını oluştururken, S proteini virüslerin konakçıya girişinin önde gelen aracısıdır. Anjiyotensin dönüştürücü enzim-2 (ACE-2), SARS-CoV ve SARS-CoV-2 dahil koronavirüsler için fonksiyonel bir reseptör olarak tanımlanmıştır. Viral füzyon SARS-CoV-2 enfeksiyonunun başlangıcında temel adımı oluşturmaktadır. Spike protein ve ACE-2 füzyonunu engelleyen ilaçların, renin-anjiyotensin-aldesteron sistemi üzerine etki eden ilaçların ve ekses dozda ACE-2'nin bu füzyon mekanizması üzerine etki ederek COVİD-19 tedaviside yer alabileceği düşünülmüştür. Bu bağlamda özellikle nano-boyutlu lipozomal taşıyıcılar, enfeksiyöz hastalıkların tedavisinde biyo-uyumlulukları ve hücreye benzer yapıları nedeniyle dikkat çekmektedir. Lipozomların geleneksel anti-enfektif ilaçları desteklemek için ikincil bir terapötik olarak da kullanıldığı çalışmalar bulunmaktadır. Bu derlemede virüslerin ACE-2 aracılı girişini engelleyerek hastalığın şiddetini azaltma ve tedavi etme ihtimali olan terapötik yaklaşımlar ele alınmıştır.

Anahtar Sözcükler: COVİD-19, SARS-CoV-2, lipozomlar, COVİD-19 tedavisi

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Introduction

Coronaviruses (CoVs) are enveloped, positively charged single-stranded RNA viruses that can cause infection in humans and animals. In 2003, the highly pathogenic and severe respiratory infection virus, called severe acute respiratory syndrome (SARS) virus (SARS-CoV), emerged in China and spread rapidly around the world. On December 29, 2019 Symptoms of lung infection (pneumonia) developed in four people working in a market selling seafood and live animals and other people who visited the same market on the same days in Wuhan, China. Later on researches showed that the cause of the infections was from a newly introduced coronavirus species. This virus has been named the new Coronavirus-2019 (COVID-19) (2). Later, it was officially named SARS-CoV-2 by the World Health Organization (WHO) (3). CoVs are enveloped, globular or polymorphic viruses ranging in size from 80-120 nm. It has a 5' capped, single stranded positively charged RNA genome, with 26.2-1.7 kb in size and is the longest of all RNA viruses. CoVs present a crown appearance under electron microscopy due to the presence of pin-like glycoproteins on the lipid envelope. For this reason, it was named "corona", which means crown in Latin (4).

There are four classes of CoVs called alpha, beta, gamma, and delta (5). Genomic characterization studies show that alphaCoV and betaCoVs originate from bat and rodents. Members of this virus family cause respiratory, enteric, hepatic, and neurological diseases in different animal species, including camels, cattle, cats and bats. To date, seven human CoV (HCoV) that can infect humans have been identified. Some HCoVs were detected in the mid-1960s, others more recently. Studies show that HCoVs causes common cold mainly during winter with 4%-15% prevalence. These viruses are HCoV-OC43, HCoV-HKU1, HCoV-229E and HCoV-NL63. While these viruses cause colds and upper respiratory tract infections in individuals with strong immune systems, they cause lower respiratory tract infections in immunocompromised individuals and in elder individuals (4).

The Betacoronavirus class includes severe acute respiratory syndrome (SARS) virus (SARS-CoV), Middle East respiratory syndrome (MERS) virus (MERS-CoV), and the COVID-19 causative agent SARS-CoV-2. Similar to SARS-CoV and MERS-CoV, SARS-CoV-2 attacks the lower respiratory system to cause viral pneumonia, but it may also affect the gastrointestinal system, heart, kidney, liver, and central nervous system leading to multiple organ failure. Mortality rates from SARS-CoV and MERS-CoV are 10% and 35%, respectively. Current information shows that SARS-CoV-2 is more contagious than SARS-CoV. As with other respiratory pathogens, including flu and rhinovirus, transmission of SARS-CoV-2 is believed to occur through respiratory droplets from coughing and sneezing. Aerosol delivery also occurs when exposed to high aerosol concentrations over long periods of time in closed areas. Based on research from the first cases in Wuhan, it has been observed that the incubation period from the transmission of infection to the occurrence of symptoms can be up to 2 weeks.

The SARS-CoV-2 genome is packaged in a helical nucleocapsid surrounded by a lipid bilayer (6). Like other CoVs, it is sensitive to ultraviolet rays and heat. Also, these viruses can be effectively inactivated with lipid solvents including ether (75%), ethanol, chlorine-containing disinfectants, peroxyacetic acid and chloroform, excluding chlorhexidine (4). The virus envelope contains at least three viral proteins called spike protein (S), membrane protein (M) and envelope protein (E). While M and E proteins form the structure of the virus, the S protein promotes the entrance of the virus into host cells (6).

Viral entry is based on a subtle interaction between the virus and the host cell. Infection begins with the interaction between host cell surface and S proteins of virus. After initial contact with the host cell receptor, enveloped viruses must fuse their envelope with the host cell membrane to deliver their nucleocapsids to the cytoplasm. The S protein plays a dual role in entry into the host cell by mediating receptor binding and membrane fusion (6).

The S protein contains two subunits, S1 and S2. The S1 subunit has a receptor binding domain (RBD) that interacts with the host cell receptor, angiotensin converting enzyme-2 (ACE-2), whereas the S2 subunit facilitates the fusion of the virus to the host cell to release genetic material into the cytoplasm to enable viral replication (7).

ACE-2 is a membrane-bound aminopeptidase that has a vital role in the cardiovascular system and immune system. ACE-2 has been identified as a functional receptor for CoVs, including SARS-CoV and SARS-CoV-2. SARS-CoV-2 infection is triggered by the binding of the S protein of the virus to ACE-2, which is highly expressed in the heart and lung cells' surfaces. SARS-CoV-2 mainly invades alveolar epithelial cells, causing life-threatening symptoms in respiratory systems (3). ACE-2 also regulates the protective mechanisms of the lungs. SARS-CoV-2 is also fatal as it blocks this protective mechanism (8). Cryo-EM structure analysis revealed that the binding affinity of the S protein of SARS-CoV-2 to ACE-2 was approximately 10-20 times higher than that of the S protein of SARS-CoV. Current information indicates that SARSCoV-2 is more transmissible/contagious than SARS-CoV (5).

Current Treatment of COVID-19

There is an urgent need for effective medication and vaccine for COVID-19 to alleviate the threat posed by the disease on public health and the burden of countries on health systems (9). Most treatment options has been formed with previous experiences in treating SARS-CoV, MERS-CoV and other viral diseases. Currently, the most important management strategy for severe cases consists of mechanical ventilation, ICU admission and supportive care. According to the WHO guidelines, it is treated with supportive care such as bed rest, oxygen saturation, adequate nutrition, prevention of dehydration, preservation of electrolyte and acid-base balance, antibiotics and isolation of patients uncertain or diagnosed with COVID-19 (10). According to data compiled by Milken Institute, headquartered in California, USA, 167 potential drugs, therapies and medical tools for the treatment of COVID-19 are at the pre-clinical or clinical stage. Of these, 55 are antibiotics, 22 antivirals, 14 are cell-based, 5 are RNA-based drug candidates, 66 are immune-converting

compounds, and 5 are medical devices such as blood purification filters and respiratory support devices (9). Drugs with previously known efficacy such as remdesivir, chloroquine, favipiravir and immune plasma therapy have been added to treatment protocols (10). In addition, vitamin C and ACE inhibitor supplementation to the treatment are discussed (9).

In order for a drug to be used in the treatment of a particular disease, a 4-stage clinical trials should be passed, each with the approval of the relevant national health authorities.

In phase I studies, drug candidate was tested on a small group of often healthy people (20 to 80) to judge its safety and side effects and to find the correct drug dosage.

Phase II studies aims to obtain preliminary data on whether the drug works in people who have a certain disease or condition while the safety studies, including short-term side effects are being continued.

In phase III studies the same procedure with phase II must be followed with a large number of patients. At this stage, if the safety and efficacy of the drug candidate is proven sufficiently, it gains the status of "new drug application" (NDA) and can be put on the market with the approval of the relevant health and trade institutions.

In phase IV studies, the possible adverse effects of the drug, which has been put on the market after the approval, on the wide user population are followed up and reported (9).

For the treatment of COVID-19, there is no cure with proven efficacy and safety that meets the approvel criteria. However, among the options evaluated for COVID-19 treatment, there are agents that inhibit viral replication, agents that bind directly to the virus and neutralizing antibodies, antibodies that target the host cell ACE-2 receptor or block S1, S2 and RBB (10). In this article, studies on vaccines and therapeutics developed through the ACE-2 and SARS-CoV-2 fusion mechanism were reviewed.

Potential Treatment Approach for COVID-19

There is no effective treatment for COVID-19 yet. Although the effectiveness of many antivirals available in the market for the treatment of the disease is evaluated, many new approaches are also considered. Viral fusion is the key step in the onset of SARS-CoV-2 infection. In this review, therapeutic approaches that may slow viral entry into cells and hence viral spread by preventing ACE-2-mediated entry of viruses are discussed. These approaches are listed in Table 1.

Drugs and vaccines that prevent spike protein and ACE-2 fusion

Based on the fact that ACE-2 is the SARS-CoV-2 receptor, the idea has arisen that a vaccine based on the S1 subunit protein can be developed and large-scale vaccine can be produced using cell lines (8).

Vaccines are generally classified as inactive or live attenuated viruses, virus-like particles (VLP), viral vectors, protein-based, DNA-based or mRNA-based vaccines (8). Protein-based vaccines can include the full length the S protein, S1 subunit, RBD, and/or the nucleocapsid of virus.

It may be necessary to use protein-based adjuvants or to fuse them with Fc unit. The most important feature of these formulations; high safety profile and their ability to stimulate cellular and humoral immune response. The developed formulations are currently in a preclinical study phase. VLP mimic the structure of the whole virus and can contain RBD, M and/or E proteins. These formulations are easy to manufacture because they can expressed in bactoviruses system. Also, VLP mimic the structure of the whole virus, unlike protein-based vaccines (11).

A study on recombinant vectors expressing the S protein of SARS-CoV revealed that this protein is highly immunogenic and

Table 1. ACE-2 based potential treatment approaches for COVID-19

Drugs and vaccines that prevent spike protein and ACE-2 fusion	Virus-like particles	RBD, M and E proteins. Similar to viral structure.
	Protein-based vaccines	Full length S protein, S1 subunit, M and/or E proteins.
	Neutralizing monoclonal antibodies	targets S protein epitopes.
Drugs affecting the renin-angitotensin-aldesteron-system	ACE-I and AT1R inhibitors	Its ability to reduce pulmonary inflammatory responses suggests that it may decrease mortality.
Excessive ACE-2 therapy	rhACE-2	Treatment with ACE2 may slow viral entry into cells and hence viral spread.

ACE: Angiotensin converting enzyme, RBB: Receptor binding site, RBD: Receptor binding domain

protective against SARS-CoV in hamsters. However, It didn't neutralized N, M and E proteins significantly (12). In addition, it has been reported that vaccines containing the subunit of S protein achieve higher neutralizing antibody titres and provide better protection than others (5). Vaccination is the best option to prevent the spread of infectious diseases. Vaccine-induced humoral immune responses specifically involve the production of neutralizing antibodies. Currently, no vaccine has been licensed to prevent SARS-CoV-2 infection. Only a few promising vaccines for SARS-CoV have reached Phase I clinical trials, but vaccine development has been stopped due to the cessation of the SARS outbreak. SARS-CoV and SARS-CoV-2 bind to the same host cell receptor (ACE-2) and have similar disease pathogenesis. For this reason, it was thought that it could be treated with common neutralizing antibodies, albeit limited. Current approaches to the development of SARS-CoV-2 vaccines are mostly based on the methods used for the development of SARS-CoV vaccines (13).

S protein plays an important role in developing immunity against SARS-CoV by stimulating neutralizing antibodies and T cells in the body. Therefore, it is believed that the full length or suitable fragment of the S protein is the most promising candidate for developing vaccine against CoVs.

It has been reported that other structural proteins do not affect the immunogenicity of the S protein or its binding to the ACE-2 receptor, which is a critical initiation step for the virus to reach the host cell. The ability of RBD in S protein to induce neutralizing antibody is quite high. In addition, recombinant proteins containing RBD and recombinant vectors encoding RBD can be used for the development of effective SARS-CoV-2 vaccines (7). Current vaccine development studies for SARS-CoV-2 are listed in Table 2.

Direct administration of monoclonal antibodies (mAb) can play an effective role in treating SARS-CoV-2 infected individuals. It has been observed that patients recovering from SARS create strong neutralizing antibody responses. S protein epitopes and functions could be targeted. With these antibodies to increase humoral protection against CoVs. RBD-specific neutralizing mAbs developed for SARS-CoV are thought to be able to provide cross-neutralization for SARS-CoV-2 due to similarity in RBD of both virus. Cross-neutralizing effect of mAbs specific to SARS-CoV RBD can be evaluated for efficacy against SARS-CoV-2 (7).

The entry of SARS-CoV into the host cell continues with the formation of hexagonal helix regions by repeating heptad (HR1 and HR2), showing structural changes in the S protein S2 domain following the binding of the S1 domain of the S protein to a receptor. Studies have shown that peptides derived from the HR2 region can inhibit SARS-CoV entry. In one study, two recombinant proteins were designed with recombinant DNA technology, one containing two HR1 and one HR2 peptide (indicated by HR121), the other containing two HR2 and one HR1 peptide (designated HR212). These two proteins exhibited high inhibitory effect on the entry of HIV/SARS pseudovirus into the host cell with IC50 values of 4.13 and 0.95 μM, respectively. In addition, these proteins have low production costs and can be easily purified. These properties suggest that HR121 and HR212 may be potent inhibitors of SARS-CoV entry (14). This has led to the idea that it can be effective in the treatment of COVID-19.

The technique based on directly isolating the antibody and designing suitable antibodies without cloning the gene previously developed for HIV treatment is also used for SARS-CoV-2 in China. Since the virus can easily develop resistance in treatment

Table 2. Current vaccine development studies for SARS-CoV-2 [Adapted from reference (10)]

Vaccine unit	Vaccine unit	Company	Progress
Protein subunit	S protein	WRAIR/USAMRIID	Preclinical
Protein subunit	S protein (Baculovirus production)	Sanofi Pasteur	Preclinical
Protein subunit	Stabilized S protein	University of Queensland/GSK	Preclinical
Protein subunit	S1 or RBD	Baylor College of Medicine	Preclinical
Protein subunit	Full Length S protein, S1, RBD, nucleocapsid • formulated with adjuvants or fused with fc	(Novavax, Phase III) recombinant S protein (Vaxine Pty Ltd, Australia, Phase I)	Preclinical
Protein subunit	S protein (Adenovirus production)	Johnson & Johnson (Jansen): In partnership with Biomedical Advanced Research and Development Authority (Barda)	Preclinical
Protein subunit	Peptite	Vaxil bio	Preclinical

with a single antibody, different combinations of mAbs should be added to these formulations (15).

Therapeutic Potential of Drugs Acting on the Renin-angiotensin-aldosteron-system

The interaction sites between ACE-2 and SARS-CoV have been defined at the atomic level, and studies so far have shown that the same principles apply to the interactions between ACE-2 and SARS-CoV-2. It was thought that by blocking these interaction sites with antibodies or small molecules, transfection of the virus could be prevented (8). Therefore, it has been suggested that ACE inhibitors such as lisinopril can be used (16).

In the lungs, activation of the local pulmonary Renin Angiotensin System (RAS) may affect the pathogenesis of lung injury by multiple mechanisms such as increased vascular permeability and alveolar epithelial cells. Angiotensin type-1 (AT1R) receptor activation provides RAS activation. It begins with renin, the starting enzyme of the pulmonary RAS activation cascade. Renin breaks down angiotensinogen, a globular protein, to produce angiotensin I (Ang I, a decapeptide hormone). ACE then converts Ang I to Ang II (Ang II, an octapeptide hormone). Ang II activates vasoactive effects by binding to angiotensin II type I (AT1) and type II (AT2) receptors. ACE-2 is an ACE homolog and plays an important role in balancing responses initiated from ACE. ACE-2 hydrolyzes Ang I to produce Ang- (1-9). ACE-2 also hydrolyzes Ang II to form Ang- (1-7), which binds to the G-protein coupled receptor MAS to antagonize most of the Ang II mediated effects. Generally, ACE-2 functions as a regulatory enzyme by decreasing native Ang II concentrations. In the lungs, RAS activity increases ACE and Ang II concentrations, and ACE-2 activities begin to rise to regulate the balance of Ang II/Ang-(1-7) levels. High Ang II levels increase vascular permeability, leading to pulmonary edema (16).

Therefore, angiotensin receptor blockers (ARBs) have the capacity to reduce inflammation and endothelial and epithelial dysfunction in many organs. ARBs preserve the integrity of the lung endothelial barrier, which is disrupted by acute injuries of the lung caused by viruses. There is substantial clinical evidence of direct effects of ARB therapy on protection of the lung associated with pneumonia, sepsis and influenza.

It was observed that the mortality rate was lower in patients treated with ARBs for cardiovascular diseases and later hospitalized for pneumonia. In studies conducted in rodents in cerebral malaria, which is caused by endothelial dysfunction, increased proinflammatory cytokine production, and increased coagulation and complement activation, ARB treatment has been observed to decrease mortality. In addition, treatment with ARBs appears to significantly reduce mortality during the Ebola epidemic in Africa, although these reports have not been fully confirmed (17).

In acute respiratory distress syndrome mouse models, ACE-2 knockout mice exhibited more severe symptoms, while overexpression of ACE-2 had some protective effects. In SARS-CoV infection of mice, it has been shown that both viral replication and viral S protein selectively reduce ACE-2 rather than reducing ACE (18). In addition, SARS-CoV also causes the rapid down-regulation of ACE-2 from the cell surface and the release of catalytically active ACE-2 ectodomains. These results show that the balance between physiological ACE/ACE-2 and angiotensin II/angiotensin (1-7) is possibly disrupted by SARS-CoV viral infection (16). Since SARS-CoV-2 binds to ACE-2, just like SARS-CoV, it is thought that this virus disrupts the RAS balance and causes exacerbation of pneumonia. Therefore, it is thought that ACEI and AT1R inhibitors may decrease SARS-CoV-2 induced pulmonary inflammatory responses and thus decrease mortality (7). There are many ongoing clinical studies in this context. In addition, there are literature suggesting that increasing the ACE-2 level of ARBs facilitates the binding of the SARS-CoV-2 virus to these regions, but the definitive information has not been obtained yet (17).

Excessive Amounts of ACE-2 as a Therapeutic Agent

Studies has begun on the idea that increasing ACE-2 activity by exogenous administration of ACE-2 may also be beneficial in human diseases with pathologically high Ang-8. As a first step, pharmacokinetics, pharmacodynamics, safety and tolerability of recombinant ACE-2 (rhACE-2) in healthy volunteers were determined. rhACE-2 was administered intravenously to healthy human subjects in a randomized, double-blind, placebo-controlled, single-dose, dose-escalation study followed by an open-label multi-dose study. ACE-2 concentrations were determined by measuring ACE-2 activity and ACE-2 content in plasma samples. Concentrations of the angiotensin system effector peptides Ang1-8, Ang1-7 and Ang1-5 were determined using liquid chromatography-tandem mass spectrometry method. As a result, single rhACE-2 doses of 100-1,200 g/kg were observed to have a dose-dependent increase in systemic exposure with biphasic elimination and a dose-independent terminal half-life (10 hours). In all single dose cohorts, Ang1-8 decreased within 30 minutes after infusion, Ang1-7 was either increased (100 and 200 lg/kg doses), or decreased or unchanged (400-1,200 lg/kg doses). Ang1-5 was transiently increased for all doses studied. Except for the lowest rhACE-2 dose, reduction in Ang1-8 levels lasted at least 24 hours. Repeated dosing (400 lg/kg for 3 or 6 days) resulted in only minimal ACE-2 accumulation and Ang1-8 levels could be suppressed over the entire administration period. As a result rhACE-2 administration was well tolerated by healthy human subjects. Despite marked changes in angiotensin system peptide concentrations, no cardiovascular effects were observed in healthy volunteers (19).

Khan et al. (20) reported the results of a phase II study examining the safety and efficacy of GSK2586881, a rhACE-2, in patients with acute respiratory syndrome. They have shown that the administration of a wide variety of GSK2586881 doses is safe without causing significant hemodynamic changes. The use of twice-daily infusion doses of GSK2586881 caused a rapid decrease in plasma Ang II levels and an increase in Ang 1-7 and Ang 1-5 levels, as well as a decrease in plasma interleukin-6 concentrations (20). Kuba et al. (18) showed that the S protein of SARS-CoV binds to ACE-2 (but not ACE) in mice,

causing severe lung injury. With the administration of ACE-2 to the lungs, the virus is likely to bind to ACE-2, which is given excessively to the body, rather than to the body's ACE-2 receptors. In this case, ACE-2 receptors that provide hemeostasis of the lung will be able to continue their functions. Thus, treatment with a soluble form of ACE2 itself may exert dual functions: (1) slow viral entry into cells and hence viral spread and (2) protect the lung from injury (8,18). Based on these ideas, it was thought that exogenous administration of ACE-2 could be therapeutic, and a clinical study was initiated to investigate the efficacy of rhACE-2 in soluble form for SARS-CoV-2. An open-label, controlled, randomized, pilot study was designed to obtain biological, physiological and clinical data about the treatment of COVID-19 patients with rhACE-2.

It is aimed to examine all biological, physiological and clinical data to determine whether there is any signal of efficacy requiring a Phase 2B trial or to determine toxic effects that would prevent such treatment. Efficacy analysis will be performed for patients receiving at least 4 doses of therapy, and safety analysis will be performed for all patients receiving at least one dose of treatment. It is expected that there will be at least 12 patients in each group. For 7 days, the experimental group will be treated 0.4 mg/kg of rhACE-2 and standard care, while the control group will be treated with only standard care (21). The result of the research will provide very important data for the treatment of COVID-19.

Nanoparticular Drug Carrier Systems

Drug delivery systems have several advantages over traditional drug forms. With these systems, the active substance can be targeted to the desired body part, thus minimizing or side effects on vital tissues. The accumulation of therapeutic compounds at the target site can be increased, resulting in more effective treatment at lower doses. This modern form of treatment is particularly important when there is a discrepancy between the dose or concentration of a drug and its therapeutic consequences or toxic effects. Cell specific targeting can be accomplished by entrapping drug in designed carriers. Various nanostructures have been tested as drug delivery systems, including liposomes, polymers, dendrimers, silicon or carbon materials, and magnetic nanoparticles (22).

Lipid or polymer-based nanoparticular drug delivery systems can be designed to improve the pharmacological and therapeutic properties of parenterally administered drugs. These particulate nano-systems have overcome the problems that prevent the clinical applications of some anticancer and antifungal drugs and have been approved for clinical use. These include lipid-based carriers such as liposomes and micelles, emulsions and lipid-drug complexes; there are also various ligand targeted products such as polymer-drug conjugates, polymer microspheres and immunoconjugates (23).

Drugs evaluated for the treatment of COVID-19 need to be administered in a drug delivery system, especially in the treatment of this disease, which causes lung involvement. Liposomes can be targeted actively or passively to the lungs (24). In addition, liposomes have been used as a trap in the treatment of viral diseases because of their cell-like properties (25,26). Liposomes could be used in COVID-19 treatment due to their biocompatibility, targeting to the region and their ability to create a trap structure.

Liposomes

Liposomes are generally defined as spherical vesicles with particle sizes ranging from 30 nm to several micrometers. They consist of a lipid bilayer surrounding the aqueous units, with the polar head groups oriented towards the inner and outer aqueous phases. These vesicles, which have one or more phospholipid bilayer membranes, may carry aqueous or lipid drugs. Liposomes were first described in the mid-1960s. Liposomes are very useful markers and tools in many disciplines such as mathematics, theoretical physics, biophysics, chemistry, colloid science, biochemistry and biology. Due to their biocompatibility, biodegradability, low toxicity, and ability to entrap both hydrophilic and lipophilic drugs, many studies have been conducted on liposomes to reduce drug toxicity and/or targeting systems. Liposomes as drug carriers has some advantages such as increased solubility of lipophilic and amphiphilic drugs, passive targeting to immune system cells, especially mononuclear phagocytic system cells, ability to deliver systemically or locally of drugs, improved bioavailability of hydrophilic molecules, and improved penetration into tissues (27).

Due to recent advances in liposome technology, many liposome-based drug formulations have been approved for clinical use. Entrapment of drugs in liposomes has provided the opportunity to improve the therapeutic effect of various agents, mainly through changing their biodistribution (28).

Research into the interaction between pathogens and liposomes began after the first mechanical studies discoverd by Bangham et al. (29) that streptolysin-S can regulate the cationic permeability of multilayer liposomes. Researchers have used liposomes as pathogen substrates. Liposome microarrays can be used for ultrasensitive detection of various types of pathogens, including viruses, bacteria and fungi. These cell membrane mimics, formed with functional ligands as well as synthetic lipids, can promote the formation of pathogen-liposome complexes that effectively increase detection sensitivity in various electrochemical and immunological assays. Another example that utilizes the liposome–pathogen interaction is direct inhibition of the pathogen infection with liposomes. Pathogens can be trapped and retained by liposomes that mimic the cell membrane, preventing them from attacking their cellular targets. In viral and bacterial infection models, treatment with functionalized liposomes has been shown to increase subject survival and reduce overall infection. Engineered liposomes have also been used as a secondary therapeutic to supplement conventional anti-infective drugs. Systemic administration of nanoscale liposomes with penicillin effectively protected animals from septicemia caused by *S. pneumoniae* and *S. aureus*. In addition, researchers have used liposome-pathogen interaction for drug release triggered by infection. Surface engineering of liposomes with specific

molecule may further increase the applicability of synthetic liposomes against infectious diseases (25,26).

Lactoferrin (LF) has strong antiviral activity against RNA and DNA viruses, including human immunodeficiency virus, zika virus, hepatitis C, cytomegalovirus, herpes simplex virus, and human papilloma virus. Serrano et al. (30) designed a prospective observational study in 75 patients with positive immunoglobulin (Ig)M/IgG tests and typical COVID-19 symptoms. In order to provide maximum anti-inflammatory and immunomodulatory effect and to protect LF from the harmful effects of enzymes and acids in the stomach, a formula encapsulated in LF phosphatidylcholine-based liposomes has been used. The patients were isolated at home and examined remotely twice a day for 10 days and followed for up to 1 month. As a dietary supplement, liposomal bovine LF (LLF) nutritional syrup (32 mg LF/10 ml + 12 mg vitamin C) was administered orally at 4 to 6 doses per day for 10 days. In addition, zinc solution was supplemented orally at a dose of 10 mg/10 mL two or three times a day. The control group received only oral LLF. In addition, half of the LLF dose given to patients' was orally administered to the patients relatives. The treatment provided a complete and rapid recovery in all patients (100%) within the first 4-5 days. In addition, COVID-19 findings were not diagnosed in the relatives of the patients. As a result of the study, the researchers concluded that LLF potentially prevented and treated COVID-19 infection (30).

It has been demonstrated in vitro studies that hydroxychloroquine (HCQ), an antimalarial and anti-inflammatory drug, inhibits SARS-CoV-2 infection and its therapeutic efficacy has been evaluated with clinical studies. In order to show its antiviral activity in vivo, it must be present in the lung at a concentration of approximately 6.7 μg/mL. However, this concentration may not be achieved with the currently recommended oral dosing regimen. Administration of high doses of HCQ to achieve the desired lung concentration raises concerns about systemic toxicity, including cardiotoxicity. It has been proven that inhaled liposomal formulations can reach effective concentrations in the lung and show efficacy at lower doses compared to systemic administration.

Tai, Wu (31) demonstrated the pharmacokinetics of inhaled liposomal HCQ in a rat model. It has been observed that respirable liposomal HCQ achieves the desired antiviral efficacy by administering it less frequently and at low doses. Therefore, the hypothesis that the formulation could be a potential treatment option in COVID-19 was supported (31).

Targeting Liposomes to the Lungs

Liposomes are widely used drug carriers by inhalation due to their safety and ability to provide controlled drug release in the lung. These carriers can entrap a wide variety of therapeutic molecules for delivery to peripheral airways in large volumes using medical nebulizers (31).

Drugs investigated for pulmonary administration via liposomes include anticancer agents (ara-C), antimicrobials (enviroxime, amikacin, pentamidine), peptides (glutathione), enzymes (superoxide dismutase), antiasthmatic and antiallergic compounds (metaproterensteol, salbutamol, sodium bichrome, corticosteroids) and promising developments such as pulmonary delivery of immunomodulators, antiviral agents and gene structures (cystic fibrosis, α 1-antitrypsin gene) are also discussed. Finally, pulmonary deposition and kinetics of drugs administered in the form of liposomal aerosols and targeting strategies for selectively delivering drugs to infected or impaired phagocytic (alveolar macrophages) and non-phagocytic (epithelial) cells in the lung are studied (32).

Liposomes could be use to reduce some of the problems in traditional aerosol administration, such as facilitating intracellular delivery of drugs, particularly to alveolar macrophages. As a result, liposomes prevent local irritation of lung tissue and reduce pulmonary toxicity; prolonging local therapeutic drug levels. So it can produce high intracellular drug concentrations in infected alveolar macrophages (32).

With passive targeting, the liposomes are delivered to the targeted areas. by controlling the size of the particles (33).

Generally, liposomes with a particle size greater than 5 μm can be passively captured by the vascular network of the lung to achieve lung targeting effect (34). Pulmonary delivery of drugs has disadvantages such as; the need for training for patients to coordinate the breathing of aerosols, the rapid absorption of most drugs, frequent dosing that often causes systemic side effects, poor water solubility of drugs that can cause local irritation and inflammation of the airways or prevent the use of aerosols completely and poor cytosolic penetration of the drug (32).

In addition, some unfavorable conditions were observed in the administration of nanoparticles to the pulmonary system via inhaler. For example, some nanoparticles can also be localized in organs distal to the respiratory tract, which can initiate the interaction of nanoparticles with subcellular structures following endocytosis by different target cells (34).

The intravenous route is a common method of delivering higher doses of drugs into the body. Many drugs loaded into carrier systems such as microspheres, microcapsules, liposomes and nanoparticles can be delivered directly to the circulation, avoiding first pass metabolism, and with the aid of these drug carriers, drugs can be delivered intravenously to the lungs. In addition, these systems can increase the drug concentration in the lung to therapeutic level after intravenous administration while reducing its distribution to other organs or tissues. At the same time, these systems can control drug release into the lungs and extend the duration of action to improve the therapeutic effect and patient compliance. Pulmonary administration in aerosol form is an interesting area of research for local or systemic delivery of the drug. However, most drugs should be applied at least three to four times a day due to their short half life.

Another disadvantage is that inhaled drugs cannot be easily delivered to the spesific area in the lungs due to the blocking of the airways from inflammation or mucus plugs, which can lead to more accumulation in the airways (34).

Result

With the information obtained from SARS and MERS outbreaks, it is known that an S protein-based vaccine could provide the required immunity. In S protein-based vaccine and drug development studies, the principle of blocking affinity of the S protein to ACE-2 has been discussed.

It has been suggested that ARBs prevent lung damage and reduce mortality due to their lung protective effect. However, since ARBs increase the rate of ACE-2, there are concerns that SARS-CoV-2 entry to host cells could increase. However, ACE-2 regulation is a very complex mechanism, and its direct positive or negative effect on SARS-CoV-2 viral spread has not yet been proven. The current clinical data mostly includes a few patients and there is no control group, so it is not possible to make a scientifically definitive result.

Developing S protein-based vaccine and treatment options by blocking the ACE-2 receptor, and proving its efficacy needs long time. However, the competitive binding of ACE-2, which is in the body's own hemostasis, to viruses after administration seems to be both a simple and effective treatment option. The results of the clinical studies conducted in this context are thought to be promising.

Drug delivery systems have several advantages over traditional drug forms. With these systems, the active substance could be targeted to the desired area, thus increasing its effect on vital tissues and minimizing side effects. Lipid or polymer-based nanoparticular drug delivery systems could be designed to improve the therapeutic effects of parenterally administered drugs.

Drugs evaluated for the treatment of COVID-19 need to be administered in a drug delivery system to reach therapeutic concentrations. Liposomes can be targted actively or passively to the lungs. In addition, liposomes have been used as a trap in the treatment of viral diseases because of their cell-like properties.

In summary, no corona virus-specific therapeutic agent, monoclonal antibodies, or vaccine has been approved. Thus, there is an urgent need for effective medication and vaccine for COVID-19 disease to limit the transmission in the community. Most treatment options for COVID-19 taken from previous experiences in treating SARS-CoV, MERS CoV, and other viral diseases. Due to the fact that SARS-CoV-2 enters the host cell through ACE-2, the basic mechanism of the drugs developed to block the affinity between ACE-2 and the S protein is mentioned in this review. In addition, liposomes based, potential drug development studies in the treatment of COVID-19 was mentioned.

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References

1. Akbabaa M, Kurtb B, Nazlıcanc E. A New Coronavirus Outbreak: MERS-CoV. Turk J Public Health 2014;12:217-27.
2. KLİMUD. Yeni Koronavirüs (''Novel Coronavirus" 2019-nCoV) - KLİMUD Klinik Viroloji Çalışma Grubu Bilgi Notu 2020. Available from: https://www.klimud.org/content/779/yeni-koronavirus-novel-coronavirus-2019-ncov---klimud-klinik-viroloji-calisma-grubu-bilgi-notu-.
3. Zheng Y-Y, Ma Y-T, Zhang J-Y, Xie X. COVID-19 and the cardiovascular system. Nat Rev Cardiol 2020;17:259-60.
4. Cascella M, Rajnik M, Cuomo A, Dulebohn SC, Di Napoli R. Features, evaluation and treatment coronavirus (COVID-19). Statpearls: StatPearls Publishing; 2020.
5. Liu C, Zhou Q, Li Y, Garner LV, Watkins SP, Carter LJ, et al. Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases. ACS Cent Sci 2020;6:315-31.
6. Belouzard S, Millet JK, Licitra BN, Whittaker GR. Mechanisms of coronavirus cell entry mediated by the viral spike protein. Viruses 2012;4:1011-33.
7. Dhama K, Sharun K, Tiwari R, Dadar M, Malik YS, Singh KP, et al. COVID-19, an emerging coronavirus infection: advances and prospects in designing and developing vaccines, immunotherapeutics, and therapeutics. Hum Vaccin Immunother 2020;16:1232-8.
8. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Med 2020;46:586-90.
9. FIP. COVID-19: Klinik Bilgi ve Tedavi Kılavuzları. 2020. Available from: https://www.fip.org/file/4825
10. Belete TM. A review on Promising vaccine development progress for COVID-19 disease. Vacunas. Amsterdam: Elseiver; 2020.
11. Vaduganathan M, Vardeny O, Michel T, McMurray JJ, Pfeffer MA, Solomon SD. Renin–angiotensin–aldosterone system inhibitors in patients with Covid-19. N Engl J Med 2020;382:1653-9.
12. Salvatori G, Luberto L, Maffei M, Aurisicchio L, Roscilli G, Palombo F, et al. SARS-CoV-2 SPIKE PROTEIN: an optimal immunological target for vaccines. J Transl Med 2020;18:1-3.
13. Shih H-I, Wu C-J, Tu Y-F, Chi C-Y. Fighting COVID-19: A quick review of diagnoses, therapies, and vaccines. Biomed J 2020;43:351-4.
14. Ni L, Zhu J, Zhang J, Yan M, Gao GF, Tien P. Design of recombinant protein-based SARS-CoV entry inhibitors targeting the heptad-repeat regions of the spike protein S2 domain. Biochem Biophys Res Commun 2005;330:39-45.
15. Sempowski GD, Saunders KO, Acharya P, Wiehe KJ, Haynes BF. Pandemic preparedness: developing vaccines and therapeutic antibodies for COVID-19. Cell 2020;181:1458-63.

16. Wu Y. Compensation of ACE2 function for possible clinical management of 2019-nCoV-induced acute lung injury. Virol Sin 2020;35:256-8.
17. Saavedra JM. Angiotensin receptor blockers and COVID-19. Pharmacol Res 2020;156:104832. doi: 10.1016/j.phrs.2020.104832. PubMed PMID: 32304747; PubMed Central PMCID: PMCPMC7158830.
18. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury. Nat Med 2005;11:875-9.
19. Haschke M, Schuster M, Poglitsch M, Loibner H, Salzberg M, Bruggisser M, et al. Pharmacokinetics and pharmacodynamics of recombinant human angiotensin-converting enzyme 2 in healthy human subjects. Clin Pharmacokinet 2013;52:783-92.
20. Khan A, Benthin C, Zeno B, Albertson TE, Boyd J, Christie JD, et al. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. Critical Care 2017;21:1-9.
21. Yimin L. Recombinant Human Angiotensin-converting Enzyme 2 (rhACE2) as a Treatment for Patients With COVID-19 2020. Available from: https://clinicaltrials.gov/ct2/show/study/NCT04287686.
22. Wilczewska AZ, Niemirowicz K, Markiewicz KH, Car H. Nanoparticles as drug delivery systems. Pharmacol Rep 2012;64:1020-37.
23. Allen TM, Cullis PR. Drug Delivery Systems: Entering the Mainstream. Science 2004;303:1818-22.
24. Chono S, Fukuchi R, Seki T, Morimoto K. Aerosolized liposomes with dipalmitoyl phosphatidylcholine enhance pulmonary insulin delivery. J Control Release 2009;137:104-9.
25. Rao L, Tian R, Chen X. Cell-membrane-mimicking nanodecoys against infectious diseases. ACS Nano 2020;14:2569-74.
26. Hendricks GL, Velazquez L, Pham S, Qaisar N, Delaney JC, Viswanathan K, et al. Heparin octasaccharide decoy liposomes inhibit replication of multiple viruses. Antiviral Res 2015;116:34-44.
27. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, et al. Liposome: classification, preparation, and applications. Nanoscale Res Lett 2013;8:102.
28. Mansoori Agrawal MA, Jawade S, Khan S, M I. A review on liposome. Int J Adv Res Pharm Bio Sci 2012;2:453.
29. Bangham A, Standish M, Weissmann G. The action of steroids and streptolysin S on the permeability of phospholipid structures to cations. J Mol Biol 1965;13:253-IN28.
30. Serrano G, Kochergina I, Albors A, Diaz E, Oroval M, Hueso G, et al. Liposomal lactoferrin as potential preventative and cure for COVID-19. Int J Res Health Sci 2020;8:8-15.
31. Elhissi A. Liposomes for pulmonary drug delivery: the role of formulation and inhalation device design. Curr Pharm Des 2017;23:362-72.
32. Schreier H, Gonzalez-Rothi RJ, Stecenko AA. Pulmonary delivery of liposomes. J Control Release 1993;24:209-23.
33. Tüylek Z. İlaç Taşıyıcı Nanosistemler. Arşiv Kaynak Tarama Derg 28:184-92.
34. Wei Y, Zhao L. Passive lung-targeted drug delivery systems via intravenous administration. Pharm Dev Technol 2014;19:129-36.

Review

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Is high Dose Intravenous Vitamin C Safe to Use in SARS-CoV-2 Treatment?

SARS-CoV-2 Tedavisinde Yüksek Doz İntravenöz C Vitamini Kullanımı Güvenli midir?

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ABSTRACT

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) disease which occurred in China in late 2019 and caused pandemia is an important public health problem. The virus has been found to be a member of the beta-coronavirus family of the same species as the SARS-CoV and SARS-related bat CoV's. The way it spreads indicates that SARS-CoV-2 can be transmitted from person to person and be more contagious than SARS-CoV. In general, SARS-CoV-2 is an acute disease, but it can be fatal and its mortality is around 2-3%. Acute respiratory distress syndrome (ARDS) is the most important cause of death. Due to cytokines storm, hyper-inflammation is a distinctive feature of ARDS, leading to cellular injury, organ failure and death. A fast, accessible, effective and safe treatment is required to save lives and reduce spreading. However, there is still no vaccine or drug developed for the prevention or definitive treatment of SARS-CoV-2. In addition to broad-spectrum antivirals and some other substances for the treatment of the disease, high-dose intravenous (i.v.) vitamin C (VC) is also recommended to take advantage of the antiviral and antioxidant effect. However, it has a pro-oxidant effect rather than an antioxidant. The cumulative effect of oxidative stress caused by inflammation and VC, besides the antiviral effect, can cause serious inflammation and oxidative damage to the tissues. In this review, the function, antiviral efficacy and possible negative consequences of high dose i.v. VC recommended for SARS-CoV2 treatment will be discussed in the light of the literature.

Keywords: SARS-CoV-2 treatment, vitamin c, intravenous, pro-oxidant, antioxidant

ÖZ

2019 sonlarında Çin'de ortaya çıkan şiddetli akut solunum sendromu koronavirüs-2 (SARS-CoV-2) hastalığı, pandemiye neden olan çok önemli bir halk sağlığı sorunudur. Virüsün, SARS-CoV ve SARS ile ilgili, yarasa CoV ile aynı türlerde, beta-koronavirüs ailesinin bir üyesi olduğu tespit edilmiştir. Bulgular, SARS-CoV-2'nin kişiden kişiye damlacık veya temas yoluyla bulaşabileceğini ve bulaşıcılığının SARS-CoV'den daha fazla olduğunu göstermektedir. SARS-CoV-2 akut geçen bir hastalık olmakla birlikte, yaşlılarda ve eşlik eden hipertansiyon gibi kronik hastalığı olanlarda daha ölümcül olabilmekte, mortalite oranı %2-3 dolayında seyretmektedir. Hastalıkla birlikte görülen akut solunum sıkıntısı sendromu (ARDS) en önemli ölüm nedenidir. Hastalıkta, sitokin fırtınasına bağlı artmış enflamasyon ve oksidatif stres, hücresel hasar ve organ yetmezliği, ölüme yol açan ARDS'nin ayırt edici özelliğidir. Hastalarda hayat kurtarmak için hızlı, erişilebilir, etkili ve güvenli tedavi yöntemlerine ihtiyaç duyulmaktadır. Bununla birlikte, SARS-CoV-2 hastalığının önlenmesi veya kesin tedavisi için geliştirilmiş bir aşı veya ilaç henüz bulunamamıştır. Hastalığın tedavisine yardımcı olabilecek geniş spektrumlu antiviral veya antimalaryal ilaçlar yanında, antiviral veya antioksidan etkisinden yararlanılmak üzere, yüksek doz intravenöz (i.v.) C vitamini (CV) kullanımı da önerilmektedir. Ancak, yüksek doz i.v. CV uygulaması, doza bağımlı olarak antioksidan etkiden ziyade, pro-oksidan etki ile ciddi oksidatif stres artışına neden olabilmektedir. Hastalarda görülen şiddetli enflamasyon ve yüksek doz CV'nin neden olduğu oksidatif stres, kümülatif olarak, antiviral etki yanında dokularda ciddi enflamasyon artışına oksidatif hasara neden olabilmektedir. Bu derlemede SARS-CoV-2 tedavisi için önerilen yüksek doz i.v. CV'nin pro-oksidan fonksiyonu, antiviral etkinliği ve doza bağımlı istenmeyen olası sonuçları literatür ışığında tartışılacaktır.

Anahtar Sözcükler: SARS-CoV-2 tedavisi, vitamin C, intravenöz, pro-oksidan, antioksidan

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Introduction

Coronaviruses (CoV) are single-chain, positive polarity, enveloped RNA viruses that cause a variety of diseases, from the common cold to more serious diseases such as the Middle East respiratory syndrome-CoV (MERS-CoV) and severe acute respiratory syndrome CoV (SARS-CoV) (1). The virus, which first appeared in the city of Wuhan in Hubei province of China in 2019 and spread all over the World and caused a pandemia, is in the sub-genus of the *Sarbecovirus,* a beta-CoV type including SARS-CoV and MERS-CoV, and has been named as SARS-CoV-2 by the World Health Organization (2). The mechanism of infection is the binding of the virus to the membrane-bound form of angiotensin converting enzyme-2 (ACE-2) and the proliferation of the complex by being taken into the cell by the host cell (3). The time from exposure to SARS-CoV-2 to the onset of symptoms (incubation time) is 2 to 14 days on average. SARS-CoV-2 infection causes severe acute respiratory disease with fever, cough and shortness of breath in many confirmed people. The organ in which SARS-CoV-2 is most effective is the lung, and the virus goes to the lungs through the respiratory tract, binds to ACE-2 receptors on the cell surfaces of the alveoli in the lung, enters the cell and multiplies inside the cell, and causes cell damage and serious inflammation. Acute inflammation caused by the virus can lead to acute respiratory distress syndrome (ARDS), which can be fatal (3). The disease is accompanied by neutrophil infiltration in the lungs, immune suppression, severe hypoxemia, and hyperferritinemia due to cytokine storm (4). Rapid production of free oxygen species (ROS) with the mechanism induced by inflammation and known as respiratory burst is the main characteristic of ARDS, which causes significant oxidative stress, cell damage, organ failure and death (5). The virus is more mortal in the elderly and those with chronic diseases such as hypertension, cardiovascular diseases and diabetes, and the mortality rate is around 2-3% (6).

Currently, a drug specific to SARS-CoV-2 has not been developed. Because viruses use normal cellular metabolic pathways as opposed to bacteria and fungi to reproduce, it is very difficult to design a drug that shows antiviral activity without damaging the cells (7). Therefore, as with other viruses, besides broad-spectrum antiviral drugs, the disease is tried to be treated with symptomatic supportive therapies. One of the supportive treatments is the use of high-dose intravenous (i.v.) vitamin C (CV).

Normally, CV is a powerful antioxidant vitamin capable of delivering electrons and cleaning ROS (8). Besides that, they show a pro-oxidant effect in the presence of pharmacologically high doses of transition metals such as iron and copper (9). In fact, it has been shown in many studies that high-dose CV causes a damage in DNAs of cancer cells and their death with pro-oxidant effect, so that it can be used as an anti-cancer drug (10). In antiviral treatment, it was desired to benefit from both antioxidant and pro-oxidant effects of high-dose CVs. While some researchers suggest the use of high dose i.v. CV to kill the virus with pro-oxidant effect, some recommends the use of high-dose i.v. CV as an antioxidant for the prevention of damage due to the increased ROS production caused by infection and inflammation (13-15).

In short, in the treatment of the disease, both as a pro-oxidant to kill the virus and as an antioxidant to eliminate oxidative damage, pharmacological i.v. high dose CV is recommended. However, in case of the use of high dose i.v. CV, cumulative effect of increased oxidative stress caused by inflammation due to disease and increased ROS with pro-oxidant effect rather than antioxidant effect, depending on the dose, may cause serious inflammation and oxidative damage in the organism besides antiviral effect. In the light of the literature, this review will discuss the structure of the CV, its physiological functions, and the use of pharmacologically high doses of CV in SARS-CoV-2 treatment and possible pathological results.

Results

Biochemical and Molecular Features of Vitamin C

CV, also known as ascorbic acid, is a monosaccharide derivative and has a structure similar to glucose and other six-carbon monosaccharides. Although it can be synthesized in most organisms, *L-gulono-g-lactone oxidase*, which catalyzes the last step of L-ascorbic acid biosynthesis, is a very important vitamin that cannot be synthesized in humans due to lack of enzyme (16). When CV is taken orally, plasma and tissue concentrations are tightly controlled by at least 4 mechanisms, namely absorption, tissue accumulation, renal excretion and re-absorption in healthy people. When taken orally, absorption is reduced depending on dose and the achievable maximum plasma CV concentration is about 250 micromol/L (um/L). On the contrary, when ascorbic acid is injected i.v. or when given by i.v. infusion, pharmacological CV concentrations can be increased to 25-30 mmol/L levels (100 fold) (17).

Physiopathological Features of SARS-CoV-2 Disease

Evidence obtained to date about SARS-CoV-2 disease has shown that hyperinflammation due to cytokine storm syndrome is very common in patients with severe clinical course and ARDS is the most important cause of mortality (4). When the virus infects lung cells, as a result of activation of epithelial cells and alveolar macrophages in the airway against the virus, chemotactic chemokines such as macrophage inflammatory protein 2 (MIP-2), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), interferon gamma (IFN-γ), and interleukin- 8 (IL-8) are released (18).

As an early response to inflammation, they can cause an abnormal permanent cascade known as cytokine storm as a result of the entry of macrophage cells into the lung and excessive release of inflammatory cytokines (19). Inflammatory mediators involving the release of chemokines, ROS, and coagulation factors, along with cytokine storm, are associated with continuously stimulated signal transduction (20). NADPH oxidase (NOX) is an important enzyme that is a source of superoxide/ROS in the pathogenesis of viral infection and initiates respiratory burst, and the target is to eliminate the pathogen (21). Hydrogen peroxide (H_2O_2), which

is a cytotoxic molecule in the catalyzer of superoxide dismutase (SOD), is synthesized from superoxide radical ($O_2^{-\bullet}$) formed as a result of the reaction.

As a result of O_2^- reaction with H_2O_2 (Haber-Weiss reaction) or Fe^{2+} reaction with H_2O_2 (Fenton reaction, in the catalyzer of myeloperoxidase (MPO) enzyme of Hydroxyl radical ($HO^\cdot$), which is the most toxic radical, and chlorine (Cl^-), hypochlorous acid, which is known as bleacher, is produced (22).

$$2O_2 + NADPH \xrightarrow{NOX} 2O_2^{-\bullet} + NADP^+ + 2H^+$$

$$2O_2^{-\bullet} + 2H \xrightarrow{SOD} H_2O_2 + O_2$$

$$O_2^{-\bullet} + H_2O_2 \longrightarrow HO^\cdot + HO^- + O_2$$

$$Fe^{2+} H_2O_2 \longrightarrow HO^\cdot + HO + Fe^{3+}$$

$$H_2O_2 + Cl^- \xrightarrow{MPO} HOCl + {}^\bullet OH$$

Although the target of ROS produced with respiratory burst stimulated with infection is to kill the microorganism, excessive and long-term ROS increase can cause lung cell damage and various chronic diseases including cancer (23).

One of the most important laboratory findings of SARS-CoV-2 infection is the excessive increase in serum ferritin levels with cytokine storm. Serum ferritin levels, normally below 200 ng/mL, may increase from 500 ng/mL to thousands (24, 25).

Use of Vitamin C in the Treatment of SARS-CoV-2 Disease

Although no definitive medication has been found for the treatment of the disease, one of the symptomatic and supportive treatment approaches is the administration of i.v. CV at pharmacologic doses increasing from 2-3 gr. to 60-90 gr. There are two different approaches of researchers who advocate the treatment of the disease with CV in pharmacological doses. The first approach is that i.v. high dose CV causes ROS production with pro-oxidant effect in the presence of transition metals such as free iron and copper, and high ROS levels show antiviral effects by creating oxidative damage in virus genome, proteins and lipid structures. As a matter of fact, studies showing the antiviral activity of high-dose CV for different viruses have been reported (26,27). In an *in vitro* study Cheng et al. (28) showed that pharmacological doses of CV increase the ROS production in extracellular fluid and kill not only isolated influenza viruses but also viruses within normal human bronchial epithelial cells. Jariwalla and Harakeh (27) reported that high doses of i.v. CV (at milimolar levels) should be used in treatment as it may have an anti-viral effect on SARS-COV-2with pro-oxidant effect and. The second approach is to take advantage of the antioxidant effect of high-dose CV to prevent oxidative damage resulting from hyperinflammation in patients. Of the researchers advocating this approach, Cheng et al. (13) reported that pharmacological doses of CV, which is a powerful antioxidant, should be used to prevent oxidative damage caused by ROS produced as a result of hyperinflammation developing in association with cytokine storm in SARS-CoV-2 disease. Boretti et al. (15) suggested that the use of i.v. CV in the treatment of SARS-CoV-2 may be effective in stopping cytokine storm.

CV at physiological levels is also involved in the regeneration of fat-soluble vitamin E, as a powerful water-soluble antioxidant that has the potential to purify ROS and reactive nitrogen species (RNS) in biological fluids by giving two electrons with direct effect (29).

In addition to its strong antioxidant feature, CV has a coenzyme property for many enzymes in the groups of monooxygenases, dioxygenases and hydroxylases in the synthesis of collagen, carnitine and proteoglycans (30). It is also known that CV contributes to immune defense by supporting various cellular functions of both natural and adaptive immune system (31). However, high doses of CV show the pro-oxidant effect in the presence of free iron (Fe^{3+}) and copper (Cu^{2+}), and cause the production of O_2^- and OH radicals, which are among the most important ROS components, with the Fenton and Heber-Weiss reactions (32).

Ascorbate (AH^-) + Fe^{3+} or Cu^{2+} ⟶ Semidehydroascorbate (A^-) + Fe^{2+} or Cu^{1+}

Free Fe^{2+} + or Cu^{1+} that occurs causes ROS production by a reaction known as the Fenton reaction.

$$Fe^{2+} \text{ or } Cu^{1+} + H_2O_2 \longrightarrow Fe^{3+} \text{ or } Cu^{2+} + OH^- + OH^\cdot$$

or

$$Fe^{2+} \text{ or } Cu^{1+} + O_2 \longrightarrow Fe^{3+} \text{ or } Cu^{2+} + O_2^-$$

$$Fe^{3+} \text{ or } Cu^{2+} + O2^{\bullet-} \longrightarrow Fe^{2+} \text{ or } Cu^{1+} + HO^\bullet + OH^-$$

As a matter of fact, studies on the use of pharmacological doses of CV as an anti-cancer drug in cancer treatment are being carried out by benefiting from the pro-oxidant feature of CV at pharmacological doses and a phase I/IIa study on the use of i.v. high-dose CV in pancreas cancer has been reported (33).

Ascorbate can be transported to the cell cytoplasm either through passive glucose transporters GLUT-1,2,3 and 4 in the form of dehydroascorbate or via sodium dependent ascorbate transporters (SVCTs) (34). In particular, as a result of SVCTs mediated transport, CV concentration in some tissues may be 100 times higher than in the outer of cell (8). It is known that cancer cells consume a lot of glucose (Warburg effect) because it uses glucose as an energy source and generates energy from glucose with anerobic metabolism (35). Because CV is more intense in cancer cells than in other cells since the molecular structure of CV is similar to glucose and uses GLUTs in the entry into cell, it can show more cytotoxic effect in cancer cells with selective pro-oxidant effect (10,36). When a high dose of i.v. CV is administered in SARS-CoV-2 infection, unlike cancer, it does not have a tissue or cell to concentrate on, and because of its

high ability to release iron from ferritin, which is very high in infection, CV produces a higher level of ROS with pro-oxidant effect. Indeed, *in vitro* and *in vivo* studies have shown that CV releases iron from ferritin and significantly increases the free iron ratio (37,38). In physiological concentrations, the contribution of CV to the release of iron from ferritin is quite low, but its pharmacological high doses have been found to have a high contribution to releasing iron from ferritin (39). In addition to the oxidative stress and high ferritin levels caused by cytokine storming in the vast majority of patients with SARS-CoV-2, pro-oxidant activity caused by high-dose i.v. CV may cause further increase of oxidative stress with cumulative effect and oxidative stress may show antiviral effect by making oxidative damage on virus protein and lipid and especially RNAs. As a matter of fact, RNAs are known to be more prone to oxidative damage than DNA (40).

In addition, high oxidative stress may also cause serious oxidative damage in the patient's protein lipids and DNA as well as antiviral effect. As a matter of fact, studies showing that high-dose CV causes oxidative damage in cells with pro-oxidant effect have been conducted. Duarte et al. (41) showed that the physiological concentration of 100 mikromol/L (um/L) VC did not cause DNA damage in fibroblast cells, but at a concentration above 100 mikromol/L (um/L), it caused DNA damage depending on dose. As the cause of the damage, it has been shown that H_2O_2, which occurs as a result of autooxidation of CV outside the cell, enters the cells by passive diffusion and increases ROS production with pro-oxidant activity as a result of reaction with intracellular free iron. With the use of high-dose i.v. CV, which is recommended for the treatment of SARS-COV-2, plasma levels of 10-20 mmol/L, which are 100-200 times higher than the normal level, can be reached (42). ROS which is produced due to inflammation, as well as increased ROS production with pro-oxidant activity, may cause cumulative high oxidative stress formation. It is known that oxidative stress can cause lipid peroxidation (43), protein oxidation (44), cell death with DNA damage, mutations, genomic instability and serious chronic diseases including cancer (45). However, in SARS-COV-2 disease, administration of CV as an antioxidant at a dose that can neutralize high levels of ROS caused by inflammation due to cytokine storm may be beneficial in treatment. However, preclinical and clinical dose optimization studies are required for the appropriate dose.

Conclusion

Since there is still no specific treatment method with proven reliability and effectiveness for SARS-CoV-2 disease, which affects the whole world, many studies are being conducted to find an effective drug. However, due to the urgency of the current situation and the limited scientific data, some treatment options with limited data on their effectiveness are suggested or used for SARS-CoV-2 disease. One of these treatments is the administration of pharmacological dose of i.v. CV. Although CV is a powerful antioxidant vitamin in physiological doses, it can increase ROS production in pharmacological doses by showing pro-oxidant effect in the presence of metals such as free iron and copper. In addition to ROS in which endogen is produced in association with the stimulation of high levels of inflammation in SARS-CoV-2 disease, ROS produced due to pro-oxidant activity caused by high CV levels together with high ferritin levels may cause cumulatively excessive ROS increase. The resulting ROS may have antiviral effects but may also cause serious oxidative damage in the patient. Therefore, before the use of high dose i.v. CV as either antiviral or antioxidant in the treatment of SARS-CoV-2 disease, pre-clinical and clinical studies should be done to optimize the dose to be applied.

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References

1. McIntosh K, Perlman S. Coronaviruses, including severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, Updated Edition 8th ed. Philadelphia, PA: Elsevier Saunders; 2015.
2. Lai C-C, Shih T-P, Ko W-C, Tang H-J, Hsueh P-R. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and corona virus disease-2019 (COVID-19): the epidemic and the challenges. Int J Antimicrob Agents 2020;55:105924.
3. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. Intensive Care Med 2020;46:586-90.
4. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet 2020;395:1033-4.
5. Hecker L. Mechanisms and consequences of oxidative stress in lung disease: therapeutic implications for an aging populace. Am J Physiol 2018;314:L642-53.
6. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 2020;8:420-422.
7. Jasim S, Naji M. A Review Novel Antiviral Agents: A Medicinal Plant Perspective, 2003. http. www blackwell-synergy com/doi/pdf/101046/j:1365-2672.2003.
8. Du J, Cullen JJ, Buettner GR. Ascorbic acid: chemistry, biology and the treatment of cancer. Biochimic Biophys Acta 2012;1826:443-57.
9. Osiecki M, Ghanavi P, Atkinson K, Nielsen LK, Doran MR. 2010. The ascorbic acid paradox. Biochem Biophys Res Commun 400:466-70.
10. Lee SJ, Jeong J-H, Lee IH, Lee J, Jung JH, Park HY, et al. Effect of high-dose vitamin C combined with anti-cancer treatment on breast cancer cells. Anticancer Res 2019;39:751-8.
11. Erol A. High-dose intravenous vitamin C treatment for COVID-19. (Dergi bilgisine ulaşamadım researchgate olabilir, ama emin değilim)2020 Preprint.
12. Pawar AY. Combating devastating COVID-19 by drug repurposing. Int J Antimicrob Agents 2020;56:105984.

13. Cheng RZ. Can early and high intravenous dose of vitamin C prevent and treat coronavirus disease 2019 (COVID-19)? Med Drug Discov 2020;5:100028.
14. Wang L, Wang Y, Ye D, Liu Q. A review of the 2019 Novel Coronavirus (COVID-19) based on current evidence. Int J Antimicrob Agents 2020;55:105948.
15. Boretti A, Banik BK. Intravenous Vitamin C for reduction of cytokines storm in Acute Respiratory Distress Syndrome. PharmaNutrition 2020:100190.
16. Nishikimi M, Fukuyama R, Minoshima S, Shimizu N, Yagi K. Cloning and chromosomal mapping of the human nonfunctional gene for L-gulono-gamma-lactone oxidase, the enzyme for L-ascorbic acid biosynthesis missing in man. J Biol Chem 1994;269:13685-8.
17. Levine M, Padayatty SJ, Espey MG. Vitamin C: a concentration-function approach yields pharmacology and therapeutic discoveries. Adv Nutr 2011;2:78-88.
18. Doherty PC, Turner SJ, Webby RG, Thomas PG. Influenza and the challenge for immunology. Nat Immunol 2006;7:449-55.
19. Perrone LA, Plowden JK, García-Sastre A, Katz JM, Tumpey TM. H5N1 and 1918 pandemic influenza virus infection results in early and excessive infiltration of macrophages and neutrophils in the lungs of mice. PLoS Pathog 2008;4:e1000115.
20. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. New York: Springer; 2017:517-28.
21. Maeda H, Akaike T. Oxygen free radicals as pathogenic molecules in viral diseases. Proc Soc Exp Biol Med 1991;198:721-7.
22. Vlahos R, Selemidis S. NADPH oxidases as novel pharmacologic targets against influenza A virus infection. Mol Pharmacol 2014;86:747-59.
23. Li N, Parrish M, Chan TK, Yin L, Rai P, Yoshiyuki Y, et al. Influenza infection induces host DNA damage and dynamic DNA damage responses during tissue regeneration. Cell Mol Life Sci 2015;72:2973-88.
24. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395:1054-62.
25. Cron RQ, Chatham WW. The rheumatologist's Role in Covid-19. J Rheumatol 2020;47:639-42.
26. Colunga Biancatelli RML, Berrill M, Marik PE. The antiviral properties of vitamin C. Milton Park, UK: Taylor & Francis; 2020.
27. Jariwalla RJ, Harakeh S. Antiviral and immunomodulatory activities of ascorbic acid. In: Subcellular biochemistry New York: Springer; 1996: 215-31.
28. Cheng LL, Liu YY, Li B, Li SY, Ran PX. 2012. [An in vitro study on the pharmacological ascorbate treatment of influenza virus]. Zhonghua Jie He He Hu Xi Za Zhi 35:520-3.
29. Sharma MK, Buettner GR. Interaction of vitamin C and vitamin E during free radical stress in plasma: an ESR study. Free Radic Biol Med 199314:649-53.
30. LeBlanc JG. Introductory Chapter: Vitamin C. In: Vitamin C-an Update on Current Uses and Functions. London; IntechOpen: 2019.
31. Carr AC, Maggini S. Vitamin C and immune function. Nutrients 2017;9:1211.
32. Boatright WL. Oxygen dependency of one-electron reactions generating ascorbate radicals and hydrogen peroxide from ascorbic acid. Food Chem 2016;196:1361-7.
33. Polireddy K, Dong R, Reed G, Yu J, Chen P, Williamson S, et al. High dose parenteral ascorbate inhibited pancreatic cancer growth and metastasis: mechanisms and a phase I/IIa study. Sci Rep 2017;7:1-15.
34. Harrison FE, May JM. Vitamin C function in the brain: vital role of the ascorbate transporter SVCT2. Free Radic Biol Med 2009;46:719-30.
35. Liberti MV, Locasale JW. The Warburg effect: how does it benefit cancer cells? Trends Biochem Sci 2016;41:211-8.
36. Franqui-Machin R, Xu H, Yethava Y, Frech I, Tricot GJ, Zhan F. Multiple Myeloma Tumor Cells Are Selectively Killed By Pharmacologically-Dosed Ascorbic Acid. Blood 2017;130 (Suppl 1):5391.
37. Bienfait H, Van Den Briel M. Rapid mobilization of ferritin iron by ascorbate in the presence of oxygen. Biochimic Biophysic Acta 1980;631:507-10.
38. Badu-Boateng C, Naftalin RJ. Ascorbate and ferritin interactions: Consequences for iron release in vitro and in vivo and implications for inflammation. Free Radic Biol Med 2019;133:75-87.
39. Badu-Boateng C, Pardalaki S, Wolf C, Lajnef S, Peyrot F, Naftalin RJ. Labile iron potentiates ascorbate-dependent reduction and mobilization of ferritin iron. Free Radic Biol Med 2017;108:94-109.
40. Li Z, Wu J, DeLeo CJ. 2006. RNA damage and surveillance under oxidative stress. IUBMB life 58:581-8.
41. Duarte TL, Almeida GM, Jones GD. Investigation of the role of extracellular H2O2 and transition metal ions in the genotoxic action of ascorbic acid in cell culture models. Toxicol Lett 2007;170:57-65.
42. Carr AC, Cook J. Intravenous vitamin C for cancer therapy–identifying the current gaps in our knowledge. Front Physiol 2018;9:1182.
43. Gaschler MM, Stockwell BR. 2017. Lipid peroxidation in cell death. Biochem Biophys Res Commun 482:419-25.
44. Møller IM, Rogowska-Wrzesinska A, Rao R. Protein carbonylation and metal-catalyzed protein oxidation in a cellular perspective. J Proteomics 2011:74:2228-42.
45. Kryston TB, Georgiev AB, Pissis P, Georgakilas AG. Role of oxidative stress and DNA damage in human carcinogenesis. Mutat Res Mutagenesis 2011;711:193-201.

Review

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Natural Alkaloids as Potential Anti-Coronavirus Compounds

Potansiyel Anti-Koronavirus Bileşikler Olarak Doğal Alkaloidler

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ABSTRACT

Coronaviruses (CoVs) are causative agents of the last three epidemics/pandemic; severe acute respiratory syndrome CoV (SARS-CoV), Middle East respiratory syndrome-CoV (MERS-CoV) and the last one SARS-CoV-2. Although meta-analysis of treatment studies against these three CoVs found no clear benefit of any spesific regimen, currently, remdesivir and favipiravir are promising potential therapies for SARS-CoV-2. On the other hand, since natural products have always played a crucial role in drug discovery and development process against various diseases, many groups in the world, are now trying to find new or repurposed natural or naturally originated drugs against viruses and CoVs. Secondary metabolites of the plants, particularly alkaloids and terpenoids have been exhibited strong antimicrobial and anticancer activities besides synthetic drugs and other natural compounds (nucleosides and nucleotides and bacterial and fungi originated ones). The first isolated secondary metabolites have been converted into important drugs since 1800's such as morphine, codeine, cocaine, and quinine have alkaloid skeleton as well as some of the recent anticancer drugs vinblastine, vincristine, taxol, etc. This review includes the last two decades of publications about natural alkaloids rather than their plant extracts which showed some promising results against CoVs. Marine organisms are also another rich source to discover new lead drugs, however they were excluded in the present review article.

Keywords: Antiviral, alkaloids, coronaviruses, SARS-CoV-2, COVID-19, natural products

ÖZ

Şiddetli akut solunum sendromu koronavirüs (SARS-CoV), Orta Doğu solunum sendromu-CoV (MERS-CoV) ve en son SARS-CoV-2 olmak üzere CoV'ler son üç epidemi/pandeminin nedeni olan ajanlardır. Fakat, henüz bu üç CoV'ye karşı spesifik bir tedavi edici ajan mevcut değildir. Doğal ürünler, çeşitli hastalıklara karşı ilaç keşfi ve geliştirilmesi sürecinde her zaman önemli bir rol oynadıkları için şu anda dünyada pek çok grup virüsler ve özellikle koronavirüslere karşı doğal veya doğal orijinli ilaç etkin maddesi bulmak için çalışmaktadır. Bitkilerin sekonder metabolitleri, özellikle alkaloidler ve terpenoidler güçlü antimikrobiyal ve antikanser aktiviteler göstermektedirler, tabii ki sentetik ve diğer doğal ilaçların yanısıra (nukleozidler, nükleotidler ve bakteriyel ve mantar orijinli bileşikler). 1800'lerden itibaren morfin, kodein, kokain ve kinin gibi ilk izole edilmiş bileşikler ve 21. yy'da vinblastin, vinkristin, taksol gibi ilaca dönüşmüş bileşiklerin hepsi alkaloid yapısına sahiptir. Bu derleme, koronavirüslere karşı umut verici bazı sonuçlar göstermiş olan alkaloidleri içeren son 20 yıldaki yayınları kapsamaktadır. Deniz organizmaları yeni aday ilaçları keşfetmek için zengin bir kaynak oluşturmasına rağmen bu derlemede hariç tutulmuşlardır.

Anahtar Sözcükler: Antiviral, alkaloidler, koronavirüsler, SARS-CoV-2, COVID-19, doğal bileşikler

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Introduction

Viral infections are known as one of the major causes of deaths worldwide, especially serious outbreaks during the last 50 years. Coronaviruses (CoVs) belonging to the Family Coronaviridae, subfamily Coronavirinae are large (genome size 26-32 kb) enveloped, positive- sense single- stranded RNA viruses which can infect both animals and humans (1-6). The sizes of pleomorphic particles of the CoVs ranged between 80-150 nm in diameter (7) and their entire replication cycle takes place in the cytoplasm. CoVs can be subdivided into four genera: alpha-, beta-, gamma-, and delta-CoVs (2, 3, 8-11), and may cause a number of diseases and even death in animals and humans, and α- and β-CoVs infect only mammals. Until now, seven CoVs have been identified as human susceptible viruses. Among them, the HCov-229E and HCoV-NL63 are human alpha-CoVs while H-CovOC43 and HCoV-HKU1 beta-CoVs. The two of them, HCoV-229E and HCoV-OC43 have been identified in 1960 which caused mild-serious cold symptoms. The HCoV-HKU1 was determined in a clinical sample from an adult with severe pneumonia in Hong Kong as a mice-originated β-coronavirus in 2005. In fact, the above mentioned four strains showed low pathogenicity causing mild respiratory symptoms. The novel virus, due to highly similar properties to the SARS-CoV which appeared with a fatal acute respiratory syndrome in 2003 (12), was named SARS-CoV-2 by World Health Organisation on February 11, 2020. The three CoVs SARS-CoV, MERS-CoV, and SARS-CoV-2 are β-CoVs which led to severe and potentially fatal respiratory tract infections. In addition, enteric, hepatic and neurological problems and lethal pneumonia were observed in COVID-19 patients (13).

The new virus (SARS-CoV-2) with a single-stranded positive-sense RNA genome is 74.5%-99% identical to that of SARS-CoV. SARS-CoV-2, as a recombinant virus is originated from bats and is transmitted to humans, possibly using an intermediate host, such as the pangolin. The SARS-CoV-2 spike protein directly binds with the host cell ACE-2 receptor facilitating virus entry and replication. It is known that viruses, without an independent enzyme system, can mutate easily exhibiting some changes in virulence, antigens, and resistance (13-15).

The physicians couldn't use a standardized treatment in the beginning due to the observation of the varied symptoms of each patient of COVID-19, and they have mainly provided supportive care. As observed previously in SARS and MERS patients, a cytokine storm has occurred in some COVID-19 patients, even more strongly as a result of an overreaction of the immune system which could be linked a redox imbalance and oxidative stress. Therefore, due to lack of enough experience, the physicians have tried to treat patients with some antiviral and anti-inflammatory drugs in general, and immunosuppressive agents, if necessary.

In 2014, an Food and Drug Administration (FDA)-approved compounds library identified four small molecules against MERS-CoV includes chloroquine (Figure 7), chlorpromazine (Figure 1), loperamide (Figure 2), and lopinavir (Figure 3) inhibiting of MERS-CoV replication in the micromolecular range (15).

However, a strategy and approaches to drug discovery and therapeutic options for CoVs specifically regarding on the key genomic elements of SARS and MERS (4,16,17) were published in 2016 included antivirals ribavirin, or additionally lopinavir-ritonavir (Figure 3), and according to the severity of

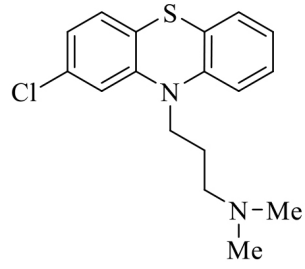

Figure 1. Chlorpromazine

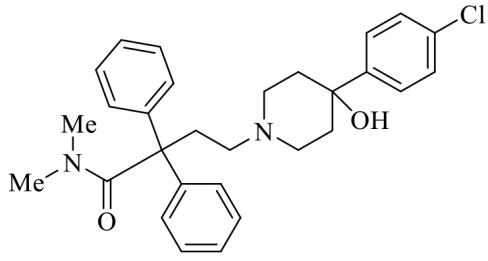

Figure 2. Loperamide

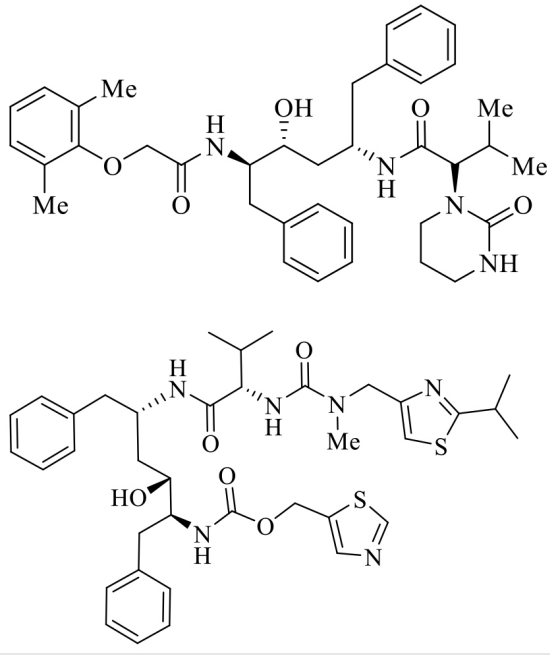

Figure 3. Lopinavir and ritonavir

the case, use of interferon combination with corticosteroids, and the convalescent plasma were used, if necessary. Any drugs or biologics have not been yet approved by the FDA for the treatment of COVID-19. However, remdesivir (Figure 4) was obtained emergency use authorization by the FDA on May 1st, 2020, based on preliminary results to the recovery of hospitalized patients with severe diseases in a shorter time. Favipiravir (Figure 5) and a few other repurposing drugs were used in China and Japan first, and they are now going to be experienced by the other countries' physicians to treat COVID-19.

The first applied drugs for the treatment of viral infections were interferons -α, -β, -γ, and ribavirin (Figure 5) in cyclophilin inhibitors as indicated by Zumla et al. (4) who reported clinical features and treatment strategies of SARS and MERS besides the epidemiologic and virologic properties. Today, the drug discovery approach is based on mainly screening of the chemical libraries. This approach is being also used to find new anti-viral drugs by high-throughput screening (HTS) of many compounds which should be evaluated by detail antiviral assays. Various classes of drugs have been discovered using drug repurposing programs. In viral outbreaks, selection of candidate drugs should be made as either virus-based or host-based treatment options. The virus-based anti-CoV treatments consist of viral nucleosides, nucleotides and nucleic acids (4).

Today, there is an urgent need to rapidly identify and develop antiviral agents. Therefore, molecular docking and other computational studies may help and direct us by screening thousands of molecules within a short time to present valuable information about small molecules and natural products that inhibit important target proteins. In a very recent study, as indicated by Gyebi et al. (18) new potential antiviral drugs should be discovered and/or developed, based on the available knowledge about ligand-protein interaction of the SARS-CoV-2 and related CoVs.

The recent six months reports (Jan-July 2020) on virtual screening of antiviral drugs, a cumulative knowledge (19) has formed based on several databases and a number of studies (20, 21), covering small molecules (4,20,22,23) and natural agents (24-26). The major targets of SARS-CoV-2 are mainly viral spike proteins (27), 3-chymotrypsin-like protease ($3CL^{pro}$), 3 CL hydrolase (27), papain like protease (PL^{pro}), RNA-dependent RNA polymerase (28), envelop proteins (29), 2'-O-ribose methyltransferase (21, 30), and nonstructural protein-3 (Nsp3) (23) and nucleocapsid protein (31).

Some secondary metabolites and their derivatives possessing anti-inflammatory and anti-viral effects exhibited high binding affinity to $3CL^{pro}$ (18). Possible inhibitory role of some natural compounds including alkaloids and phenolics against the $3CL^{pro}$ of SARS-CoV-2 should be more rapidly investigated to find new natural lead compounds (32). There is an urgent need to search other sources and evaluate to obtain new compounds with antiviral activity. Because, no any drug exactly treats COVID-19, and some of the drugs used to cause several problems, namely viral resistance (33).

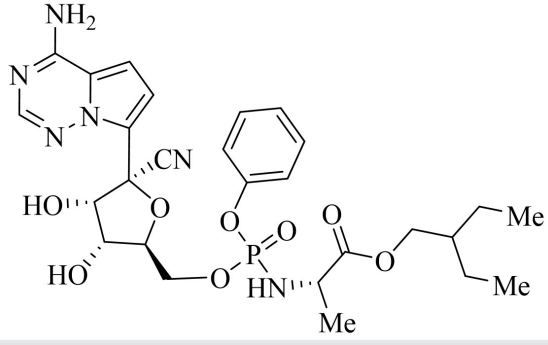

Figure 4. Remdesivir

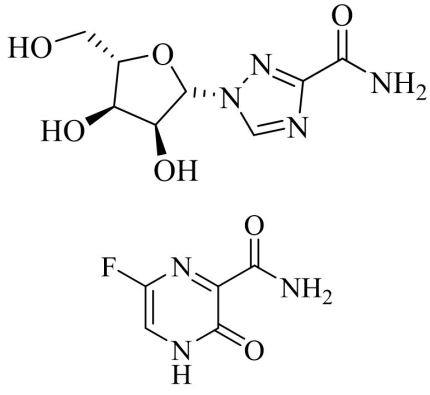

Figure 5. Ribavirin and favipiravir

History of Alkaloids Isolated From Plants as Potential Drugs

Plants and their secondary metabolites have a pivotal role in the discovery of the novel drugs and might be a good solution in finding new antiviral drugs. Alkaloids as one the bioactive secondary metabolites are well known pharmacologically, over 10,000 alkaloids being isolated from plants or bacteria/fungi and/or marine organisms. However, the number of studies carried out on their antiviral and immunomodulatory properties. Since 19th century, many secondary metabolites have been isolated from plant extracts, most of them having alkaloid skeleton, such as morphine, quinine (Figure 6), codeine, striknin, brucine, veratrine, cocaine which were converted into important drugs to treat many disorders or diseases. Among them, quinine (Figure 6) was isolated in 1818 by two French pharmacists Pelletier and

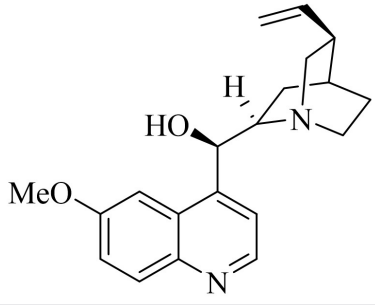

Figure 6. Quinine

Caventou and they ushered to the Scientific French Academia its isolation from *Cinchona* tree bark in 1820, and they have then presented extraction method to the drug industry without patenting to overcome malaria immediately, and then quinine (Figure 6) has been used to treat malaria until today. Its derivatives chloroquine (Figure 7) and hydroxychloroquine (Figure 8) are now used in the treatment of SARS-2 at the initial level of the disease. Therefore, alkaloids always took place as major potential compounds in the drug discovery, especially as antimicrobial, anti-inflammatory, and cytotoxic/anticancer agents. A literature survey indicates that (18,22,34-36) alkaloids may be potential antiviral agents with nitrogen-containing structures rather than other secondary metabolites. A study on the investigation of 54 medicinal plants against different viruses exhibited their antiviral potential (35).

Recent 20 Year Studies on Alkaloids as Potential Coronaviruses' Inhibitors

In the present review, we aimed to evaluate the last 20 years of studies carried out on the alkaloids as potent anti-coronaviral properties. For this purpose, first, we have searched the literature considering alkaloids isolated from the terrestrial plants which shown antiviral activity, particularly against CoVs, excluding studies on marine organisms as well as antiviral activities of the plant or animal extracts rather than their pure isolates.

A Brassicaceae family plant *Isatis indigotica* is a medicinal plant and used as a folkloric medicine. Among isolated 12 compounds from *I. indigotica* roots, three alkaloids indirubin, indican (Figure 12), indigo (Figure 10), and a glucosinolate sinigrin (Figure 9) and β-sitosterol were tested for anti-SARS-CoV $3CL^{pro}$ effects, of these compounds indigo, sinigrin, β-sitosterol inhibited cleavage actvity of the $3CL^{pro}$ in cell-free and cell-based assay. Sinigrin showed a good correlation between the effects on cell-free and cell-based cleavage of the SARS-CoV $3CL^{pro}$, and sinigrin and indigo were not found to be toxic to Vero Cells (34).

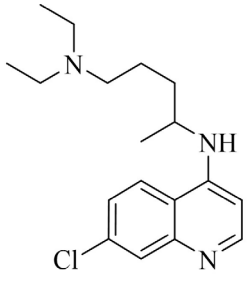

Figure 7. Chloroquine

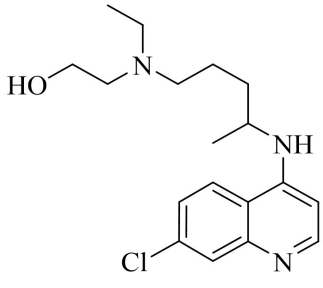

Figure 8. Hydroxychloroquine

I. indigotica also used in the clinical treatment of several viral diseases. Isolated compounds from I. indigotica (isatin, indican, indigotin and indirubin) (Figures 11 and Figure 12), exhibited immunomodulatory and antiviral effects. Another study of Chang et al. (37) on *I. indigotica* extracts and the alkaloids; indigo (Figure 10) and indirubin (Figure 12) inhibited Japanese encephalitis virus replication *in vitro*.

Binding affinities of 20 alkaloids as well as some terpenoids isolated from some African plants consist of 8 indol alkaloids, 5 naphtoisoquinoline alkaloids, 5 cryptolepine alkaloids, a furoquinoline and a diterpene alkaloid were investigated against the 3-chymotrypsin-like protease ($3CL^{pro}$) (18). As a result of the study, the two natural alkaloids 10-hydroxyusambarensine (Figure 13) and cryptoquindoline (Figure 14), were found to be potent of inhibiting both SARS-CoV-2 and SARS-CoV. Therefore, they should be subjected to advance experimental studies against SARS-CoV-2 $3CL^{pro}$ for the prevention and treatment of COVID-19.

In another comprehensive study, over 200 Chinese medicinal plant extracts were screened for antiviral activity against SARS-CoV, among them, the four extracts showed moderate to potent

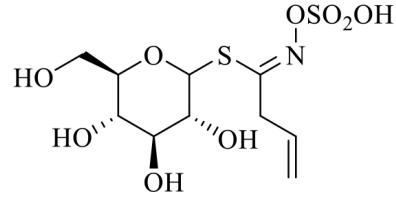

Figure 9. Sinigrin

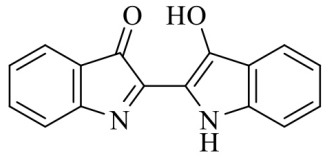

Figure 10. Indigo

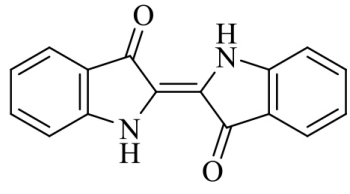

Figure 11. Indigotin

antiviral activities. From the tested four plant extracts (*Lycoris radiata, Artemisia annuna, Pyrrosia lingua, Lindera aggregata*), *L. radiata* was the most potent extract, and a pure compound lycorine (Figure 15) was identified anti-SARS-CoV alkaloid with an EC_{50} value 15.7±1.2 nM (38). In a recent study, lycorine was also investigated against several virus strains (HCoV-OC43, HCoV-NL63, MERS-CoV, and MHV-A59) inhibiting cell division with the IC_{50} values 0.15, 0.47, 1.63, and 0.31 μM, respectively (22,38,39). However, the toxic effect of lycorine at low dosage (~1mg/kg in dogs) should be considered (28).

Another study was performed in 2005 on a Chinese medicinal plant focusing to determine the anti-SARS-CoV activity of cepharanthine (Figure 16) *in vitro* (40). *Stephania tetrandra* and other related species of Menispermaceae family afforded bis-benzylisoquinoline alkaloids including cepharanthine (Figure 16), fangchinoline (Figure 17), and tetrandrine (Figure 18). Although anticancer and anti- inflammatory activities of these alkaloids were previously studied, their antiviral activity studies are still ongoing. In 2019, a study reported on the antiviral activity of bis-benzylisoquinoline alkaloids; cepharanthine, tetrandrine and fangchinoline showing

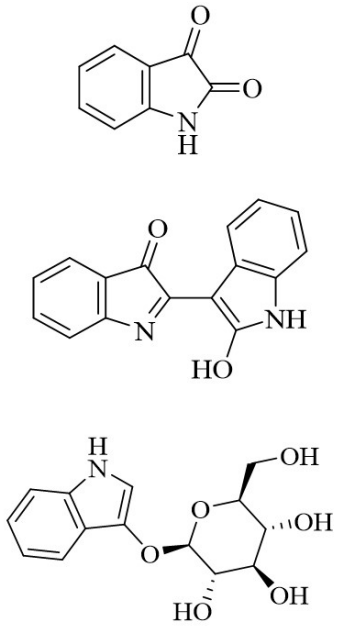

Figure 12. Isolated three alkaloids from *Isatis indigotica*

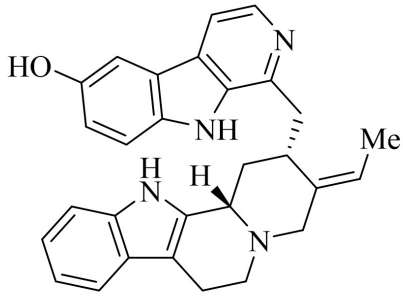

Figure 13. 10-hydroxyusambarensine

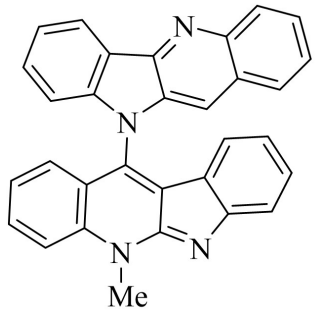

Figure 14. Cryptoquindoline

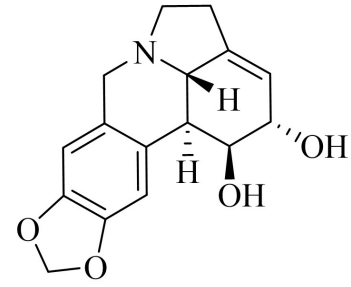

Figure 15. Lycorine

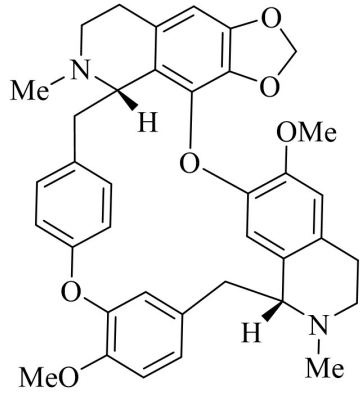

Figure 16. Cepharanthine

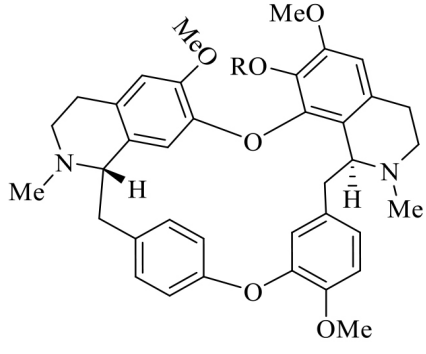

Figure 17. Fangchinoline
Figure 18. Tetrandrine

significant inhibition on virus human coronavirus HCoV-OC43-infected MRC-5 human lung cells. They subsequently suppressed its replication and inhibition of viral S and N protein expressions (41).

In a previous study, 17 alkaloids out of 49 alkaloids isolated from the methanol extract of another *Stephania* species *S. cepharantha* have shown anti herpes-simplex-virus activity (42).

Some natural and synthetic phenanthroindolizidines and phenanthroquinolizidines alkaloids were found to be potential in vitro inhibitors against enteropathogenic coronavirus transmissible gastroenteritis virus (TGEV). They also decreased cytopathic effect in Vero 76 cells infected by SARS CoV. These alkaloids, named "tylophorine compounds" (Figure 19) can be potent anti-coronavirus agents in the future to treat TGEV or SARS-CoV infections (43).

In a recent study, among 290 agents screened against the SARS and MERS CoVs, an alkaloid emetine (Figure 20) was found to be the most promising agent showing the lowest half-maximal effect. The most notable result was the observation of emetine in the human blood 300 times higher in the lungs. In fact, emetine was reported earlier as a MERS-CoV replication inhibitor and approved in 2015 by the FDA drug register list (44,45).

In a very recent study, some plant extracts and their isolates were searched against different human CoVs including the strains of SARS-CoV-2, SARS-CoV, MERS-CoV, and HCoV. Among the potent antiviral compounds, several alkaloids or alkaloid-like compounds were found to be promising agents besides some phenolics. Their action mechanisms are given below in parenthesis for each; including desmethoxyreserpine (by replication, 3CL^{pro} & entry) (Figure 21) and moupinamide (by PL^{pro}) (Figure 22) against SARS-CoV-2; sabadinine (by inhibition of CoV protease) (Figure 23), aurantiamide acetate (by inhibition of active pocket of CoV protease) (Figure 24), sinigrin (by inhibition of 3CL^{pro}) (Figure 9) and indigo (by inhibition of 3CL^{pro}) (Figure 10) against SARS-CoV 3CLpro through inhibition of CoV-protease, tryptanthrin (Figure 25) and indigodole B (Figure 26) (by blocking viral RNA genome synthesis and PL^{pro} 2 activity against viral strain HCoV-NL63) (28,34).

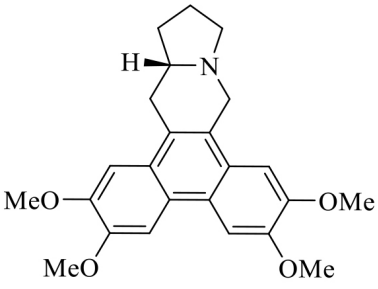

Figure 19. Tylophorine

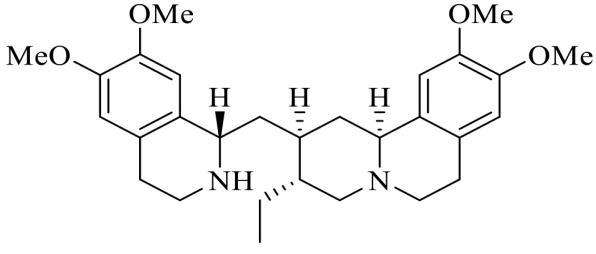

Figure 20. Emetine

Conclusion

In the 21st century, the COVID-19 pandemic poses a major challenge to mankind following 2002-2003 SARS and 2012

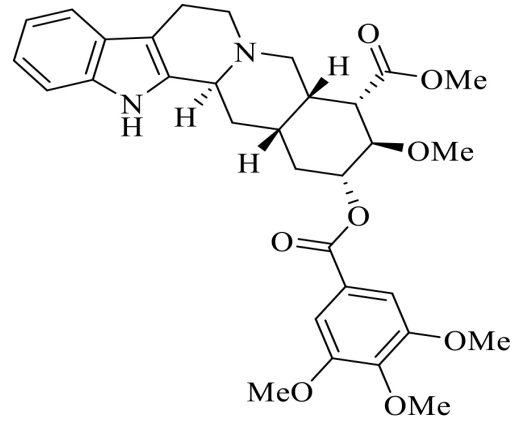

Figure 21. Desmethoxyreserpine

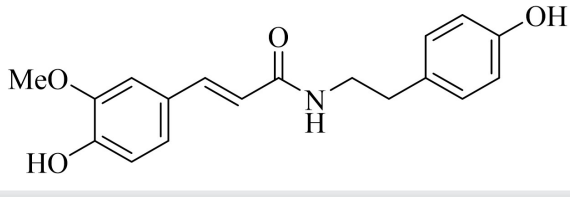

Figure 22. Moupinamide

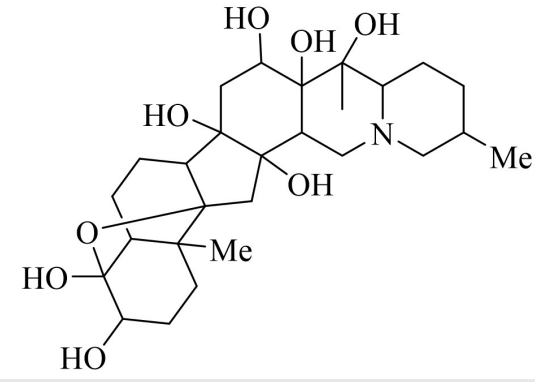

Figure 23. Sabadinine

MERS epidemics. Although a vaccine would be available in the near future, a variety of mutant forms of the CoVs would occur, particularly of the SARS-CoV-2. Therefore, we have to find new sources and new strategies to create new drugs from all possible sources. For this purpose, the nature is an unbelievable treasure with rich biodiversity of terrestrial plants and animals, as well as marine organisms which most of them have never studied yet. As a result of over the last 20 years in search of finding anti-viral drugs, some promising natural products were determined having alkaloid or terpenoid or phenolic structures. Among natural compounds tested for antiviral activity, about 100 alkaloids were found to be potent anti-viral agents, at least. This number will be increased by searching marine organisms. Microbes, especially bacteria and fungi are other resources to produce new drugs as well as nucleosides, nucleotides, and nucleic acids. Arbidol which is a small indol derivative which has been previously tried to use in the treatment of a bench of viral diseases including coronaviral ones and even now in COVID-19, but still more clinical trials are needed. Several indol alkaloids mentioned above in this review are also promising antiviral drugs. In this alarming period, as emergency agents favipravir and remdesivir (Figure 4) have been used by some countries as known antiviral compounds and they are still subjected to a large number of clinical trials. Considering literature studies and presently used and developed drugs, most of the treatment mechanisms of the viral infections require the drugs which work as protein/enzyme inhibitors, in general. In the case of COVID-19, the SARS-CoV-2 uses ACE-2 as receptor, via binding of spike glycoprotein (S protein) to ACE-2, therefore soluble ACE-2 might be a most potent candidate for COVID-19 treatment. Beside many synthetic compounds, particularly natural compounds have been shown strong inhibition on main protease ($3CL^{pro}$) of the SARS-CoV-2, which can be lead drugs. The other protease (PL^{pro}) and some other enzyme inhibitors (such as neuraminidase inhibitors) in addition to RNA-dependent RNA polymerase inhibitors are another potential drugs. Thus, in silico virtual screening studies should be the first step in discovering new and/or developing repurposing drugs from both synthetic and natural sources, then *in vitro*, *in vivo* and clinical studies should be carried out immediately.

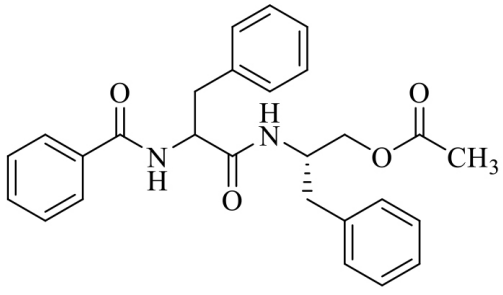

Figure 24. Aurantiamide acetate

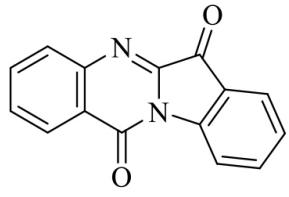

Figure 25. Tryptanthrin

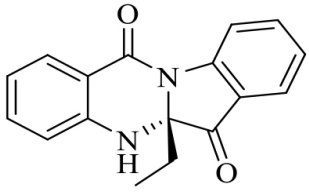

Figure 26. Indigodole B

Peer-review: Internally peer reviewed.

Authorship Contributions

Concept: , Design: , Data Collection or Processing: , Analysis or Interpretation: , Literature Search: , Writing:

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Woo PCY, Lau SKP, Huang Y, Yuen KY. Coronavirus diversity, phylogeny and ınterspecies jumping. Exper Biol Med 2009;234:1117-27.
2. Chan JFW, Li KSM, To KKW, Cheng VCC, Chen HL, Yuen KY. Is the discovery of the novel human betacoronavirus 2c EMC/2012 (HCoV-EMC) the beginning of another SARS-like pandemic? J Infect 2012;65:477-89.
3. Chan JFW, Lau SKP, Woo PCY. The emerging novel Middle East respiratory syndrome coronavirus: The "knowns" and "unknowns". J Formos Med Assoc 2013;112:372-81.
4. Zumla A, Chan JFW, Azhar EI, Hui DSC, Yuen KY. Coronaviruses - drug discovery and therapeutic options. Nat Rev Drug Discov 2016;15:327-47.
5. Li F. Structure, Function, and Evolution of Coronavirus Spike Proteins. Annu Rev Virol 2016;3:237-61.
6. Yuki K, Fujiogi M, Koutsogiannaki S. COVID-19 pathophysiology: A review. Clin Immunol 2020;215:108427.
7. Neuman BW, Kiss G, Kunding AH, Bhella D, Baksh MF, Connelly S, et al. A structural analysis of M protein in coronavirus assembly and morphology. J Struct Biol 2011;174:11-22.
8. Chan JFW, To KKW, Tse H, Jin DY, Yuen KY. Interspecies transmission and emergence of novel viruses: lessons from bats and birds. Trends Microbiol 2013;21:544-55.

9. Chan JFW, To KKW, Chen HL, Yuen KY. Cross-species transmission and emergence of novel viruses from birds. Curr Opin Virol 2015;10:63-9.
10. Woo PCY, Lau SKP, Lam CSF, Lau CCY, Tsang AKL, Lau JHN, et al. Discovery of Seven Novel Mammalian and Avian Coronaviruses in the Genus Deltacoronavirus Supports Bat Coronaviruses as the Gene Source of Alphacoronavirus and Betacoronavirus and Avian Coronaviruses as the Gene Source of Gammacoronavirus and Deltacoronavirus. J Virol 2012;86:3995-4008
11. Lau SKP, Woo PCY, Li KSM, Tsang AKL, Fan RYY, Luk HKH, et al. Discovery of a Novel Coronavirus, China Rattus Coronavirus HKU24, from Norway Rats Supports the Murine Origin of Betacoronavirus 1 and Has Implications for the Ancestor of Betacoronavirus Lineage A. J Virol 2015;89:3076-92.
12. Yi Y, Lagniton PNP, Ye S, Li EQ, Xu RH. COVID-19: what has been learned and to be learned about the novel coronavirus disease. Int J Biol Sci 2020;16:1753-66.
13. Sun PF, Lu XH, Xu C, Wang YJ, Sun WJ, Xi JN. CD-sACE-2 inclusion compounds: An effective treatment for coronavirus disease 2019 (COVID-19). J Med Virol 2020. doi: 10.1002/jmv.25804.
14. Zhang T, Wu QF, Zhang ZG. Probable Pangolin Origin of SARS-CoV-2 Associated with the COVID-19 Outbreak (vol 30, 1346.e1, 2020). Curr Biol 2020;30:1346-51.e2.
15. de Wilde AH, Jochmans D, Posthuma CC, Zevenhoven-Dobbe JC, van Nieuwkoop S, Bestebroer TM, et al. Screening of an FDA-Approved Compound Library Identifies Four Small-Molecule Inhibitors of Middle East Respiratory Syndrome Coronavirus Replication in Cell Culture. Antimicrob Agents Chemother 2014;58:4875-84.
16. Barnard DL, Kumaki Y. Recent developments in anti-severe acute respiratory syndrome coronavirus chemotherapy. Future Virol 2011;6:615-31.
17. Kilianski A, Baker SC. Cell-based antiviral screening against coronaviruses: Developing virus-specific and broad-spectrum inhibitors. Antiviral Res 2014;101:105-12.
18. Gyebi GA, Ogunro OB, Adegunloye AP, Ogunyemi OM, Afolabi SO. Potential inhibitors of coronavirus 3-chymotrypsin-like protease (3CL(pro)): an in silico screening of alkaloids and terpenoids from African medicinal plants. J Biomol Struct Dyn 2020;1-13
19. Boopathi S, Poma AB, Kolandaivel P. Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment. J Biomol Struct Dyn 2020;1-10.
20. Muralidharan N, Sakthivel R, Velmurugan D, Gromiha MM. Computational studies of drug repurposing and synergism of lopinavir, oseltamivir and ritonavir binding with SARS-CoV-2 protease against COVID-19. J Biomol Struct Dyn 2020;1-6.
21. Khan RJ, Jha RK, Amera GM, Jain M, Singh E, Pathak A, et al. Targeting SARS-CoV-2: a systematic drug repurposing approach to identify promising inhibitors against 3C-like proteinase and 2 '-O-ribose methyltransferase. J Biomol Struct Dyn 2020;1-14.
22. Islam MT, Sarkar C, El-Kersh DM, Jamaddar S, Uddin SJ, Shilpi JA, et al. Natural products and their derivatives against coronavirus: A review of the non-clinical and pre-clinical data. Phytother Res 2020;34:2471-92.
23. Pillaiyar T, Manickam M, Namasivayam V, Hayashi Y, Jung SH. an overview of severe acute respiratory syndrome-coronavirus (SARS-CoV) 3CL protease ınhibitors: peptidomimetics and small molecule chemotherapy. J Med Chem 2016;59:6595-628.
24. Aanouz I, Belhassan A, El-Khatabi K, Lakhlifi T, El-Idrissi M, Bouachrine M. Moroccan Medicinal plants as inhibitors against SARS-CoV-2 main protease: Computational investigations. J Biomol Struct Dyn 2020;1-9.
25. Elfiky AA, Azzam EB. Novel guanosine derivatives against MERS CoV polymerase: An in silico perspective. J Biomol Struct Dyn 2020:1-9.
26. Pant S, Singh M, Ravichandiran V, Murty USN, Srivastava HK. Peptide-like and small-molecule inhibitors against COVID-19. J Biomol Struct Dyn 2020;1-10.
27. Elmezayen AD, Al-Obaidi A, Sahin AT, Yelekci K. Drug repurposing for coronavirus (COVID-19): in silico screening of known drugs against coronavirus 3CL hydrolase and protease enzymes. J Biomol Struct Dyn 2020:1-13.
28. Mani JS, Johnson JB, Steel JC, Broszczak DA, Neilsen PM, Walsh KB, et al. Natural product-derived phytochemicals as potential agents against coronaviruses: A review. Virus Res 2020;284:197989.
29. Gupta MK, Vemula S, Donde R, Gouda G, Behera L, Vadde R. In-silico approaches to detect inhibitors of the human severe acute respiratory syndrome coronavirus envelope protein ion channel. Journal of Biomolecular Structure & Dynamics 2020:1-11.
30. Khan SA, Zia K, Ashraf S, Uddin R, Ul-Haq Z. Identification of chymotrypsin-like protease inhibitors of SARS-CoV-2 via integrated computational approach. J Biomol Struct Dyn 2020;1-10
31. Sarma P, Shekhar N, Prajapat M, Avti P, Kaur H, Kumar S, et al. In-silico homology assisted identification of inhibitor of RNA binding against 2019-nCoV N-protein (N terminal domain). J Biomol Struct Dyn 2020:1-9.
32. Chen YW, Yiu CB, Wong KY. Prediction of the SARS-CoV-2 (2019-nCoV) 3C-like protease (3CL (pro)) structure: virtual screening reveals velpatasvir, ledipasvir, and other drug repurposing candidates. F1000Res 2020;9:129.
33. Vijayan P, Raghu C, Ashok G, Dhanaraj SA, Suresh B. Antiviral activity of medicinal plants of Nilgiris. Indian J Med Res 2004;120:24-9.
34. Lin CW, Tsai FJ, Tsai CH, Lai CC, Wan L, Ho TY, et al. Anti-SARS coronavirus 3C-like protease effects of Isatis indigotica root and plant-derived phenolic compounds. Antiviral Res 2005;68:36-42.
35. Akram M, Tahir IM, Shah SMA, Mahmood Z, Altaf A, Ahmad K, et al. Antiviral potential of medicinal plants against HIV, HSV, influenza, hepatitis, and coxsackievirus: A systematic review. Phytother Res 2018;32:811-22.
36. Oliveira A, Teixeira RR, de Oliveira AS, de Souza APM, da Silva ML, de Paula SO. Potential antivirals: natural products targeting replication enzymes of dengue and chikungunya viruses. Molecules 2017;22:505.
37. Chang SJ, Chang YC, Lu KZ, Tsou YY, Lin CW. Antiviral activity of ısatis indigotica extract and ıts derived ındirubin against japanese encephalitis virus. Evid Based Complement Alternat Med 2012;2012:925830.

38. Li SY, Chen C, Zhang HQ, Guo HY, Wang H, Wang L, et al. Identification of natural compounds with antiviral activities against SARS-associated coronavirus. Antiviral Res 2005;67:18-23.

39. Shen L, Niu JW, Wang CH, Huang BY, Wang WL, Zhu N, et al. High-throughput screening and ıdentification of potent broad-spectrum ınhibitors of coronaviruses. J Virol 2019;93:e00023-19.

40. Zhang CH, Wang YF, Liu XJ, Lu JH, Qian CW, Wan ZY, et al. Antiviral activity of cepharanthine against severe acute respiratory syndrome coronavirus in vitro. Chinese Med J 2005;118:493-6.

41. Kim DE, Min JS, Jang MS, Lee JY, Shin YS, Park CM, et al. Natural Bis-Benzylisoquinoline Alkaloids-Tetrandrine, Fangchinoline, and Cepharanthine, Inhibit Human Coronavirus OC43 Infection of MRC-5 Human Lung Cells. Biomolecules 2019;9:696.

42. Nawawi A, Nakamura N, Meselhy MR, Hattori M, Kurokawa M, Shiraki K, et al. In vivo antiviral activity of Stephania cepharantha against herpes simplex virus Type-1. Phytother Res 2001;15:497-500.

43. Yang CW, Lee YZ, Kang IJ, Barnard DL, Jan JT, Lin D, et al. Identification of phenanthroindolizines and phenanthroquinolizidines as novel potent anti-coronaviral agents for porcine enteropathogenic coronavirus transmissible gastroenteritis virus and human severe acute respiratory syndrome coronavirus. Antiviral Res 2010;88:160-8.

44. Bleasel MD, Peterson GM. Emetine, Ipecac, Ipecac Alkaloids and Analogues as Potential Antiviral Agents for Coronaviruses. Pharmaceuticals (Basel) 2020;13:51.

45. Liu Q, Xia S, Sun Z, Wang Q, Du L, Lu L, et al. Testing of Middle East respiratory syndrome coronavirus replication inhibitors for the ability to block viral entry. Antimicrob Agents Chemother 2015;59:742-4.

Review

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Promising Potential Pharmaceuticals from the Genus Cordyceps for COVID-19 Treatment: A Review Study

COVİD-19 Tedavisi için Cordysceps Cinsinden Gelen Potansiyel İlaçlar: Bir Derleme Çalışması

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ABSTRACT

A novel coronavirus (2019-nCoV), named as severe acute respiratory syndrome-CoV-2 (SARS-CoV-2) later, was first detected at Wuhan, China, in December 2019. COVID-19, the disease caused by SARS-CoV-2, is highly contagious and has mild to severe symptoms ranging from generalized weakness, headache, dizziness, fever, cough, and dyspnea to severe hypoxia with acute respiratory distress syndrome and multiorgan failure. The unclarity of the virus' pathophysiology and lack of a targeted therapy make the disease very challenging to treat for physicians. *Cordyceps sinensis* and *Cordyceps militaris* are entomopathogenic fungi which are used for their anti-inflammatory, immunomodulatory, lung improving, and antiviral functions in Traditional Chinese Medicine. Thus, advanced search for *C. sinensis* and *C. militaris* has proven several compounds from these fungi have the functions that they are thought to have. This review aims to discuss whether *C. sinensis* and *C. militaris* can be used for COVID-19 treatment in light of previous studies.

Keywords: COVID-19, *C. sinensis*, *C. militaris*, antiviral, anti-inflammatory

ÖZ

Şiddetli akut solunum yolu enfeksiyonu-koronavirüs-2 (SARS-CoV-2) olarak adlandırılan yeni bir koronavirüs (2019-nCoV), ilk olarak Aralık 2019'da Vuhan, Çin'de tespit edildi. SARS-CoV-2'nin neden olduğu COVID-19 oldukça bulaşıcıdır ve genel halsizlik, baş ağrısı, baş dönmesi, ateş, öksürük ve nefes darlığından akut solunum sıkıntısı sendromu ve multiorgan yetmezliği olan şiddetli hipoksiye kadar hafif ile şiddetli semptomlara sahiptir. Virüsün patofizyolojisinin belirsizliği ve hedefe yönelik bir tedavinin olmaması, doktorlar için hastalığı tedavi etmeyi çok zorlaştırır. *Cordyceps sinensis* ve *Cordyceps militaris*, Geleneksel Çin Tıbbında anti-enflamatuvar, immünomodülatör, akciğer iyileştirici ve antiviral fonksiyonları için kullanılan entomopatojenik mantarlardır. Bu nedenle, *C. sinensis* ve *C. militaris* için yapılan ileri araştırmalar, bu mantarlardan elde edilen çeşitli bileşiklerin düşünülen işlevlere sahip olduklarını kanıtlamıştır. Bu derleme, daha önce yapılan çalışmalar ışığında, *C. sinensis* ve *C. militaris*'in COVID-19 tedavisi için kullanılıp kullanılamayacağını tartışmayı amaçlamaktadır.

Anahtar Sözcükler: COVID-19, *C. sinensis*, *C. militaris*, antiviral, anti-enflamatuvar

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Introduction

Coronaviruses (CoVs) are non-segmented positive-sense, single-stranded RNA viruses with an envelope belonging to the family of *Coronaviridae* (1). They are zoonotic viruses with a broad distribution among humans, other mammals, and birds; causing a wide range of infections which affect respiratory, gastrointestinal, hepatic, and nervous systems (2). Until the novel coronavirus (2019-nCoV) was first detected at Wuhan, China and spread rapidly all over the world (3), six species of the virus were known to cause human disease. While four of them [229E, OC43, NL63, and HKU1) typically cause common cold symptoms, the other two beta-CoVs (SARS-CoV (4) and MERS-CoV (5)] can cause severe acute respiratory syndrome which can be fatal (6). Later, the novel CoV (nCoV) was designated as SARS-CoV-2 due to its similarity to SARS-CoV, and the name CoV disease-19 (COVID-19) was given to the disease caused by SARS-CoV-2 (7). International Committee on Taxonomy of Viruses has announced the official name of the virus as SARS-CoV-2 (8). The typical presentation for COVID-19 is lower respiratory tract infection with fever, dry cough, and dyspnea (9). These respiratory symptoms can range from minimal symptoms to severe hypoxia with ARDS. Thus, the involvement of other organ systems and symptoms as generalized weakness, headache, dizziness, diarrhea, and vomiting are also reported (10). To the date, higher mortality rates in older adults and lower incidence in children have been reported by epidemiological studies (11). Recent studies of CoVs have shown that increased levels of serum proinflammatory cytokines are associated with pulmonary inflammation and damage in Middle East Respiratory Syndrome-CoV (MERS-CoV) (12), SARS-CoV (13), and SARS-CoV-2 (10). Previous reports of increased levels of proinflammatory cytokines such as interleukin (IL)-6, IL-8, IL-10, tumor necrosis factor (TNF)-α, granulocyte-colony stimulating factor, monocyte chemoattractant protein-1 and macrophage inflammatory protein-1α in the plasma with severe disease indicate inflammatory response and cytokine storm plays a crucial role in the disease's pathophysiology (14). Since no targeted therapy has been discovered to treat COVID-19 yet, it is still challenging for physicians to treat the disease with classic supportive techniques (rest, oxygen saturation, prevention of dehydration, adequate nutrition and control water, electrolyte and acid/base balance) (15) and antivirals (16). Nevertheless, mushrooms can be another treatment option in the light of their usage for previous SARS-CoV infection.

The number of species of mushrooms in the world is estimated to be 140,000, but only 10 % (about 14,000 named species) are known (17). Mushrooms have been consumed by humans for their nutrient and pharmaceutical properties since ancient times (18). Especially in Traditional Chinese medicine, they have been used to support the immune system, to improve lung and kidney functions, to treat chronic bronchitis (19), to control the blood glucose levels, and to protect the liver (20). Previous researches showed that mushrooms contained a vast amount of bioactive compounds such as phenolic, flavonoid, and organic compounds, terpenoids, lectins, proteins, and polysaccharides (21-23), which can be used as immunostimulatory, anti-inflammatory, antiviral, antioxidant, antifungal, and antitumoral in modern medicine (24).

The genus Cordyceps under the division of Ascomycota includes over 500 species of fungi which are entomopathogenic to arthropods (25). *C. sinensis* and *C. militaris* are the most known and the most valued species of the genus in Traditional Chinese Medicine (26). They have been used as medicine for their immunological regulation, free radical scavenger, anticancer, antiviral, antifungal, analgesic, antihyperlipidemic, antileukemic, and lung improvement properties in Traditional Chinese Medicine (27,28) (Figure 1). The exhaustive search for COVID-19 treatment has made the drugs of different purposes available for the COVID-19 treatment, indicating drugs derived from mushrooms can also be applicable for the treatment. Thus, many studies have shown that *C. sinensis* and *C. militaris* possess antiviral, immunomodulatory, and lung function protective effects, which can also be applicable for COVID-19 treatment. A previous study by Singh et al. (29) showed that *C. sinensis* increased the tolerance against hypoxia developing in the lung by increasing Nrf2 and HIF1α and decreasing NFκB *in vitro*. It also increased the anti-inflammatory cytokine TGF-β (29). Moreover, another study by Fu et al. also showed supportive results with the protective effect of *C.sinensis* extract on lipopolysaccharide-induced lung injury in mice. They demonstrated that this effect was occurred via reducing the total number of macrophages and neutrophils and expression of TNF-α, IL-6, IL-1β; the binding ability of NF-κB p65 DNA and inhibiting the mRNA expression of COX-2 and iNOS in lung tissue with a dose-dependent manner (30). Their data also revealed that *C. sinensis* could be used as a potential drug for acute lung injury treatment. Another study by Chen et al. (31) also showed similar results for *C. sinensis* with reducing bleomycin-induced fibrosis in mice by decreasing the number of inflammatory cells and fibroproliferative foci with a dose-dependent manner. Their data suggested that *C. sinensis* could be used not only to prevent lung fibrosis but also to treat it. In addition, Wang et al. (32) and Xu et al. (33) studies have shown that *C. sinensis* inhibits lung fibrosis.

These results were also supported by Kim et al. (34) with showing cordycepin from *C. militaris* inhibits the NO production in macrophages by downregulating the iNOS, COX-2 expression, and TNF-α gene expression. Additionally, Yang et al. (35) showed that cordycepin inhibited the Th2 dominated inflammation and reduced Th2 associated cytokines, including IL-4, IL-5, and IL-13, with a dose-dependent manner on Ova-induced mouse model of asthma.

In addition to their anti-inflammatory effects, *C. sinensis* and *C. militaris* also have an antiviral effect on several viruses. In 1991, Mueller et al. (36) reported that cordycepin had an antiviral effect on HIV-1 *in vitro*. Therefore, Jiang et al. (37) reported adenosine from *C. militaris* had an HIV-1 protease inhibitory effect. Lee et al. (38) demonstrated the antiviral effect of *C. militaris* on DBA/2 mice infected with H1N1. They showed a significant survival improvement with *C. militaris* treatment. They also demonstrated a significant decrease of TNF-α on treatment

group, suggesting *C. militaris* has an immune-enhancing in healthy mice while the immune-inhibitory effect in H1N1 infected mice (38). Another study for *C. militaris*' anti-influenza effect was performed by Ohta et al. (39). They demonstrated a significant decrease of virus titers in both lung tissue and bronchoalveolar lavage fluid of the mice treated with an acidic polysaccharide (APS) isolated from *C. militaris* intranasally. Since they did not observe any direct antiviral effect of the APS *in vitro*, they thought the anti-influenza effect of the APS was occurred by its immunomodulatory effects (39). In addition to anti-HIV and anti-influenza activities, *C .militaris* also has the anti-HCV effect, which was previously described by Ueda et al. (40). They also reported that cordycepin was probably the responsible molecule for this activity by inhibiting RNA-dependent RNA-polymerase (NS5B) of HCV (40).

C. *sinensis* and C. *militaris* can modulate the immune response in addition to their anti-inflammatory, antiviral, antioxidant, and antifibrotic properties mentioned in the above literature; It may be suitable for the pathologies that occur in COVID-19.

Discussion

SARS-CoV-2, which is previously known as nCoV, has spread throughout the world rapidly after it was first detected at Wuhan, China, and declared as a pandemic by WHO. Although pathophysiology of the virus has not been clarified completely yet, it is known that the cytokine storm plays a crucial role, and lungs are the most affected organs with severe inflammation. Thus, there are no data available about the disease's long term effects on the lungs and other organs. Permanent fibrosis may be seen in the patients overcoming the disease. It seems like drugs targeting the excessive immune response and the virus itself hold the potential to be used for COVID-19 treatment. *C. sinensis* and *C. militaris* are traditionally used mushrooms to improve lung functions and regulate the immune system in China. Thus,

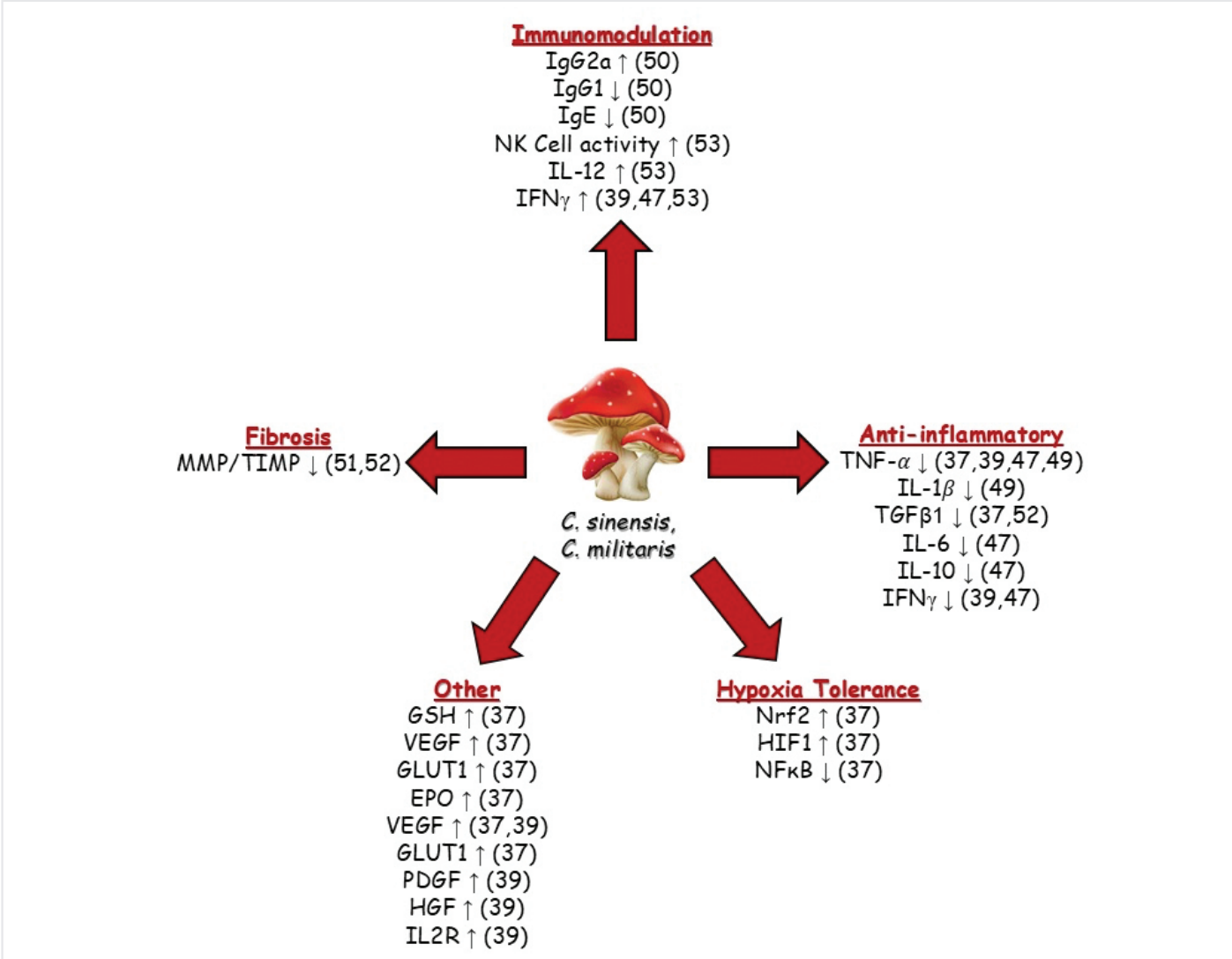

Figure 1. Effects of *C. sinensis* and *C. militaris*

Ig: Immunoglobulin, IL: Interleukin, IFN: Interferon, MMP: Matrix metalloproteinase, TIMP: Tissue inhibitors of metalloproteinases, TNF: Tumor necrosis factor, GSH: Glutathione, VEGF: Vascular endothelial growth factor

according to previous researches mentioned above, *C. sinensis* and *C. militaris* can be effective agents for the prevention and treatment of COVID-19 by immunomodulating, reducing the proinflammatory cytokines, preventing lung fibrosis, improving tolerance to hypoxemia and inhibiting the viral enzymes.

Moreover, they can also be used either to maintain the lung functions after overcoming the disease. This paper demonstrated that *C. sinensis* and *C. militaris* might be used for the treatment of the COVID-19 for reducing inflammation and fibrosis, increasing immune response and antiviral effect. It may be a better option to use anciently known and well-studied agents rather than discovering new ones to find a rapid treatment for COVID-19 in these pandemic times. Further investigations of the mechanisms involved are needed.

Limitation

While *C. militaris* can be cultured in the laboratory conditions, *C. sinensis* can only grow in the nature, and it may be endangered due to overuse. Therefore, many types of research mainly focused on *C. militaris* due to either difficulty to find *C. sinensis* samples and to protect the species from the human overuse.

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Authorship Contributions

Concept: M.A.K., E.M.G., Design: M.A.K., E.M.G., Data Collection or Processing: M.A.K., E.M.G., Analysis or Interpretation: M.A.K., E.M.G., Literature Search: M.A.K., E.M.G., Writing: M.A.K., E.M.G.

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References

1. Richman DD, Whitley RJ, Hayden FG. Clinical virology. 4th ed. Hoboken, NJ; John Wiley & Sons: 2016.
2. Seah I, Agrawal R. Can the coronavirus disease 2019 (COVID-19) affect the eyes? A review of coronaviruses and ocular implications in humans and animals. Ocul Immunol Inflamm 2020;28:391-5.
3. Bai Y, Yao L, Wei T, Tian F, Jin D-Y, Chen L, et al. Presumed asymptomatic carrier transmission of COVID-19. JAMA 2020;323:1406-7.
4. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020;382:727-33.
5. de Groot RJ, Baker SC, Baric RS, Brown CS, Drosten C, Enjuanes L, et al. Commentary: Middle East respiratory syndrome coronavirus (MERS-CoV): announcement of the Coronavirus Study Group. J Virol 2013;87:7790-2.
6. Wevers BA, van der Hoek L. Recently discovered human coronaviruses. Clin Lab Med 2009;29:715-24.
7. Yuki K, Fujiogi M, Koutsogiannaki S. COVID-19 pathophysiology: A review. Clin Immunol 2020:108427.
8. Gorbalenya AE. Severe acute respiratory syndrome-related coronavirus–The species and its viruses, a statement of the Coronavirus Study Group. BioRxiv. 2020.
9. Sohrabi C, Alsafi Z, O'Neill N, Khan M, Kerwan A, Al-Jabir A, et al. World Health Organization declares global emergency: A review of the 2019 novel coronavirus (COVID-19). Int J Surg 2020;76:71-6.
10. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506.
11. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet 2020;395:507-13.
12. Mahallawi WH, Khabour OF, Zhang Q, Makhdoum HM, Suliman BA. MERS-CoV infection in humans is associated with a proinflammatory Th1 and Th17 cytokine profile. Cytokine 2018;104:8-13.
13. Wong C, Lam C, Wu A, Ip W, Lee N, Chan I, et al. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. Clin Exper Immunol 2004;136:95-103.
14. Zhou Y, Fu B, Zheng X, Wang D, Zhao C. Pathogenic T cells and inflammatory monocytes incite inflammatory storm in severe COVID-19 patients. Natl Sci Rev 2020:nwaa041.Yang Y, Peng F, Wang R, Guan K, Jiang T, Xu G, et al. The deadly coronaviruses: The 2003 SARS pandemic and the 2020 novel coronavirus epidemic in China. J Autoimmun 2020:102434.
15. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, et al. A trial of lopinavir–ritonavir in adults hospitalized with severe Covid-19. N Engl J Med 2020;382:1787-99.
16. Wasser S. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Applied microbiology and biotechnology 2002;60:258-74.
17. Akgul H, Sevindik M, Coban C, Alli H, Selamoglu Z. New approaches in traditional and complementary alternative medicine practices: Auricularia auricula and Trametes versicolor. J Tradit Med Clin Natur 2017;6:2.
18. Wasser SP, Weis AL. Medicinal properties of substances occurring in higher basidiomycetes mushrooms: current perspectives. Crit Rev Immunol
19. 1999;19:65-96.Wani BA, Bodha R, Wani A. Nutritional and medicinal importance of mushrooms. J Med Plants Res 2010;4:2598-604.
20. Wang S, Marcone MF. The biochemistry and biological properties of the world's most expensive underground edible mushroom: Truffles. Food Res Int 2011;44:2567-81.
21. Villares A, García-Lafuente A, Guillamón E, Ramos Á. Identification and quantification of ergosterol and phenolic compounds occurring in Tuber spp. truffles. Journal of food composition and analysis 2012;26:177-82.
22. He J-Z, Ru Q-M, Dong D-D, Sun P-L. Chemical characteristics and antioxidant properties of crude water soluble polysaccharides from four common edible mushrooms. Molecules 2012;17:4373-87.
23. Gomes DCV, de Alencar MVOB, Dos Reis AC, de Lima RMT, de Oliveira Santos JV, da Mata AMOF, et al. Antioxidant, anti-

inflammatory and cytotoxic/antitumoral bioactives from the phylum Basidiomycota and their possible mechanisms of action. Biomed Pharmacother 2019;112:108643.

24. Zheng P, Xia Y, Xiao G, Xiong C, Hu X, Zhang S, et al. Genome sequence of the insect pathogenic fungus Cordyceps militaris, a valued traditional Chinese medicine. Genome Biol 2012;12:R116.
25. SP UL, Yang F, Karl W. Quality control of Cordyceps sinensis, avaluedtraditionalChinesemedicine [J1. Pharm Biomed Anal 2006;41:1571-84.
26. Zhu J-S, Halpern GM, Jones K. The scientific rediscovery of an ancient Chinese herbal medicine: Cordyceps sinensis Part I. J Altern Complement Med Fall 1998;4:289-303Jordan J, Sullivan A, Lee T. Immune activation by a sterile aqueous extract of Cordyceps sinensis: mechanism of action. Immunopharmacol Immunotoxicol 2008;30:53-70.
27. Singh M, Tulsawani R, Koganti P, Chauhan A, Manickam M, Misra K. Cordyceps sinensis increases hypoxia tolerance by inducing heme oxygenase-1 and metallothionein via Nrf2 activation in human lung epithelial cells. Biomed Res Int 2013;2013:569206Fu S, Lu W, Yu W, Hu J. Protective effect of Cordyceps sinensis extract on lipopolysaccharide-induced acute lung injury in mice. Biosci Rep 2019;39: BSR20190789Chen M, Cheung FW, Chan MH, Hui PK, Ip S-P, Ling YH, et al. Protective roles of Cordyceps on lung fibrosis in cellular and rat models. J Ethnopharmacol 2012;143:448-54.
28. Wang S, Bai W, Wang C, Dai Z. Effects of cordyceps sinensi on bleomycin-induced pulmonary fibrosis in mice. Zhongguo Zhong Yao Za Zhi 2007;32:2623-7.
29. Xu H, Li S, Lin Y, Liu R, Gu Y, Liao D. Effectiveness of cultured Cordyceps sinensis combined with glucocorticosteroid on pulmonary fibrosis induced by bleomycin in rats. Zhongguo Zhong Yao Za Zhi 2011;36:2265-70.
30. Kim HG, Shrestha B, Lim SY, Yoon DH, Chang WC, Shin D-J, et al. Cordycepin inhibits lipopolysaccharide-induced inflammation by the suppression of NF-κB through Akt and p38 inhibition in RAW 264.7 macrophage cells. Eur J Pharmacol 2006;545:192-9.
31. Yang X, Li Y, He Y, Li T, Wang W, Zhang J, et al. Cordycepin alleviates airway hyperreactivity in a murine model of asthma by attenuating the inflammatory process. Int Immunopharmacol 2015;26:401-8.
32. Mueller WE, Weiler BE, Charubala R, Pfleiderer W, Leserman L, Sobol RW, et al. Cordycepin analogs of 2', 5'-oligoadenylate inhibit human immunodeficiency virus infection via inhibition of reverse transcriptase. Biochemistry 1991;30:2027-33.
33. Jiang Y, Wong J, Fu M, Ng T, Liu Z, Wang C, et al. Isolation of adenosine, iso-sinensetin and dimethylguanosine with antioxidant and HIV-1 protease inhibiting activities from fruiting bodies of Cordyceps militaris. Phytomedicine 2011;18:189-93.
34. Lee HH, Park H, Sung G-H, Lee K, Lee T, Lee I, et al. Anti-influenza effect of Cordyceps militaris through immunomodulation in a DBA/2 mouse model. J Microbiol 2014;52:696-701.
35. Ohta Y, Lee J-B, Hayashi K, Fujita A, Park DK, Hayashi T. In vivo anti-influenza virus activity of an immunomodulatory acidic polysaccharide isolated from Cordyceps militaris grown on germinated soybeans. J Agric Food Chem 2007;55:10194-9.
36. Ueda Y, Mori K, Satoh S, Dansako H, Ikeda M, Kato N. Anti-HCV activity of the Chinese medicinal fungus Cordyceps militaris. Biochem Biophys Res Commun 2014;447:341-5.
37. Qian G-m, Pan G-F, Guo J-Y. Anti-inflammatory and antinociceptive effects of cordymin, a peptide purified from the medicinal mushroom Cordyceps sinensis. Nat Prod Res 2012;26:2358-62.
38. Hsu C-H, Sun H-L, Sheu J-N, Ku M-S, Hu C-M, Chan Y, et al. Effects of the immunomodulatory agent Cordyceps militaris on airway inflammation in a mouse asthma model. Pediatr Neonatol 2008;49:171-8.
39. Kelly EA, Jarjour NN. Role of matrix metalloproteinases in asthma. Curr Opin Pulmonary Med 2003;9:28-33.
40. Liu Y-K, Shen W. Inhibitive effect of cordyceps sinensis on experimental hepatic fibrosis and its possible mechanism. World J Gastroenterol 2003;9:529.
41. Jung S-J, Jung E-S, Choi E-K, Sin H-S, Ha K-C, Chae S-W. Immunomodulatory effects of a mycelium extract of Cordyceps (Paecilomyces hepiali; CBG-CS-2): a randomized and double-blind clinical trial. BMC 2019;19:77.

Review

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Vaccination Studies Against COVID-19 Agent: Current Status

COVİD-19 Etkenine Karşı Aşı Çalışmaları: Mevcut Durum

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ABSTRACT

The emergence of the new pathogenic viral strains is a constant threat to global health. The latest new coronavirus strain coronavirus-2019 (COVID-19) is the best-known example of this. COVID-19, caused by the severe acute respiratory syndrome-CoV-2 virus has quickly spread around the globe. The applications of drug and vaccine practices, and the identification of potential candidates are of high importance against this pandemic. On healthy adults, a proper evaluation of potential vaccines in parallel with vaccination studies on animal models and an enlargement of production capacity will benefit minimum risk and accelerated COVID-19 vaccine studies for human subjects. The purpose of this review is to contribute to the analysis of vaccine development strategies, vaccine development stages, phase studies and the difficulties, production stages and current status of potential vaccine studies developed to prevent COVID-19.

Keywords: COVID-19, vaccine, pandemic

ÖZ

Yeni patojenik viral suşların ortaya çıkması küresel sağlık için sürekli bir tehdit oluşturmaktadır. En son yeni koronavirüs-2019 (COVİD-19), bunun en iyi bilinen örneğidir. Şiddetli akut solunum yolu enfeksiyonu-koronavirüs-19 virüsünün neden olduğu COVİD-19 hızla dünyaya yayılmıştır. Bu salgına karşı ilaç ve aşı çalışmalarının gerçekleştirilmesi ve potansiyel aşı adaylarının hızlı bir şekilde tanımlanması oldukça önemlidir. Sağlıklı yetişkinlerde aday aşıların, hayvan modellerindeki aşı çalışmalarına paralel olarak doğru şekilde değerlendirilmesi ve üretim kapasitesinin büyütülmesi, insan denekler için minimum risk ve hızlandırılmış COVİD-19 aşı çalışmalarına fayda sağlayacaktır. Bu derlemenin amacı, aşı geliştirme stratejileri, aşı gelişim aşamaları, faz çalışmaları ile COVİD-19'u önlemeye yönelik geliştirilen aday aşı çalışmalarının güçlükleri, üretim aşamaları ve mevcut durumları hakkında bilgi vererek analiz edilebilmelerine katkı sunmaktır.

Anahtar Sözcükler: COVİD-19, aşı, pandemi

Introduction

In December 2019, a new coronavirus (CoV) pneumonia due to animal-to-human transmission emerged in the local wild animal markets of Wuhan, Hubei province of China, and then the virus started to spread from person to person (Figure 1) (1). nCoV-2019, called a new SARS-like CoV (SARS-CoV-2), has spread all over the world and has become a global public health problem over time. The World Health Organization (WHO) stated that the disease agent is a nCoV and declared the coronavirus disease (COVID-19) as a Public Health Emergency of International Importance on Jan 30th, 2020, and then as a pandemic on Mar 11th, 2020 (1,2).

CoVs, a large single-stranded RNA virus (+ssRNA) family that can be isolated in different animal species, have gradually become the main pathogens of the respiratory tract disease outbreaks (3). Coronaviruses (CoVs) are viruses with the largest RNA

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genome (about 120 nanometers in size) detected to date. The coronavirus genome, which is a member of the Coronaviridae family, resembles a crown sphere with positive polarity (mRNA), single-stranded, enveloped, 125 nanometer in size, wand-like projections around it (4). CoV ses that affect both humans and animals are divided into four different subgroups, alpha, beta, gamma and delta CoV, based on the sequence identity of the spike (ear of grain) protein or non-structural proteins. Human coronaviruses (HCoV) such as 229E, OC43, NL-63 and HKU-1 are highly contagious respiratory viruses responsible for approximately 10-20% of annual common cold cases. HCoV-related disease can often cause severe upper and lower respiratory tract infections in the young and elderly population (1). Highly pathogenic CoVs can arise from zoonotic reservoirs (5). It is stated that SARS-CoV-2 is inactivated by ultraviolet (UV) rays or by heating at 56 °C for 30 minutes, and is sensitive to many disinfectants such as diethyl ether, 75% ethanol, chlorine, peracetic acid and chloroform. In addition, it has been shown that SARS-CoV-2 is more permanent on plastic and stainless steel than copper and cardboard and can survive more than 72 hours on these surfaces (6).

Mechanism of Action for COVID-19

The genetic sequence of SARS-CoV-2 is ≥70% similar to SARS-CoV. For this reason, it is stated that the virus uses the same cellular entry receptor Angiotensin-converting enzyme-2 (ACE-2) (7). ACE-2, most commonly found in lung type 2 alveoli, is also expressed by many cells, including glial cells and neurons (8). After SARS-CoV-2 enters alveolar epithelial cells, it proliferates rapidly and triggers a strong immune response. Ultimately, this condition causes cytokine storm and pulmonary tissue damage (7). Cytokine storm is a very important immune system hyperreaction in which cytokine is released into the systemic circulation at a rapid and very high rate. Fever, acute respiratory distress and multiple organ failure develop due to hypercytokinemia, which is called the uncontrolled production of pro-inflammatory cytokines (7,9). SARS-CoV-2 infection reduces the total number of T-cells, CD4+ and CD8+ T-cells, and destroys surviving T-cells functionally (7). In addition, interleukin (IL-6, IL-2, IL-7, IL-10), interferon (INF), tumor necrosis factor (TNF-α), macrophage and inflammatory protein (MIP1A) levels are high and mostly coagulopathy and diffuse intravascular coagulation (DIC) develops. In severe cases, microvascular thrombosis and coagulation factors are depleted in DIC that develops as a result of the activation of fulminant coagulation (10).

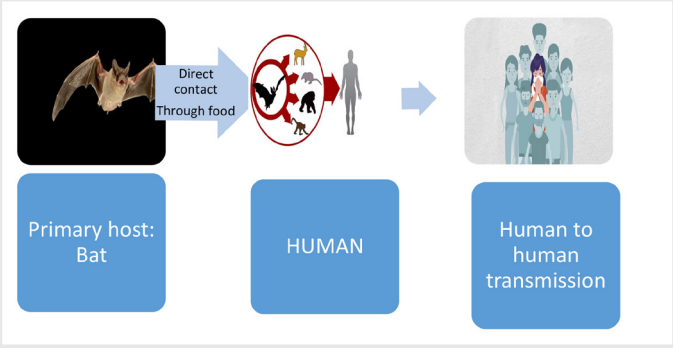

Figure 1. Severe acute respiratory syndrome coronavirus 2 transmission route

Viral Immune Response

The innate immune response is the first line of defense to protect against pathogen (virus, bacteria) infections. Pattern recognition receptors (PRR) produced by immune system cells are used to identify molecules such as double stranded viral RNA pathogen. When viruses infect the host, they multiply in many different cells of the host, settle in different tissues and show high antigenic properties in living organisms. Because of this feature, they generate both humoral and cellular immune response after they enter the organism. This developing immune response depends on the structure of the virus, the way it spreads, and the structural, genetic and biological characteristics of the host. With respect to respiratory viruses, Toll-like receptor (TLR3) 7 and 9 recognize various viral replication products. Each of the TLRs that recognize nucleic acids expressed by macrophages and dendritic cells, which are part of innate immunity, can activate the transcription of genes that induce IFN (lambda interferon). When viral infections develop type 1 IFN expression (usually IFN-α, IFN-β) and an innate antiviral defense response occurs. Antiviral activity performed by type I IFNs directly inhibits viral replication. In addition, type 1 IFNs can regulate the cellular immune functions of both natural and adaptive immune systems, and they do this to conserve long-term immunity and maintain resistance to viral infections. Among the three types of IFN, IFN-λ, one of the Type III IFNs, plays an important role in antiviral immune activities (11).

Immunity Expected as a Result of Viral Vaccines

The type of vaccines, the size, character and duration of the immune memory are important in providing immunity. T-cells, which are very important in the immune system, express the antigen-recognizing receptors and degenerate while recognizing the antigen. The selection of T-cells in the thymus is governed by the interactions of T-cell receptors. In this case, the selection of T cells is necessary for the recognition of pathogen-derived antigens (12). One mechanism defined by changes in humoral and cellular vaccine responses is the polymorphism in the major histocompatibility complex genes. Other genetic factors are polymorphisms in PRRs such as TLR or RIG-I like receptors (RLR). In addition, single nucleotide polymorphisms (SNP) in other genes and different expression levels of genes also influence vaccine responses (13). In vaccine studies on coronavirus, SARS-CoV particles inactivated by β-propiolactone, formalin, UV light or a combination of the two techniques were administered to animals (mice, rabbits) in various ways, and as a result, higher IgG antibody titers were detected as a result of high-dose vaccination. In addition, it has been determined that DNA vaccines expressing the SARS-CoV S protein protect against virus replication by inducing humoral and cellular immunity in mice, causing an increase in CD4+ T-cells and creating a stronger antibody response (14).

Vaccine Definition and Antiviral Vaccine Types

Vaccines, which have the potential to save life and protect people from the effects of infectious diseases, are biological substances "developed from pathogens or synthetically" that cause disease in order to gain immune memory by creating an immune response and to produce antibodies. The aim of vaccination is to facilitate the rapid response required to prevent disease formation (15).

Antiviral vaccines can be divided into two broad categories.

a. Gene-based vaccines: Gene-based vaccines deliver gene sequences that encode protein antigens that are produced by host cells. These include live-virus vaccines, recombinant vaccine vectors, or nucleic acid vaccines.

b. Protein-based vaccines: These vaccines include fully inactivated virus, individual viral proteins or subdomains, or viral proteins that are all assembled as particles all produced in vitro. Recombinant vaccine vectors and nucleic acid vaccines are best suited for speed because they can be more easily adapted to platform production technologies where upstream supply chains and downstream processes are the same for each product (16).

Vaccine Development Strategies

One of the important goals in vaccine development is to determine the immune response that should be elicited by the vaccine. Antibody mediated neutralization has traditionally been the main target of vaccines. Because many pathogens require receptor-mediated binding to cells and/or fusion, or mediate pathogenicity by producing specific toxins. These can all represent protective antibody targets. New vaccines targeting more complex pathogens are designed to enhance other aspects of innate and adaptive response. The relative contribution of antibodies, CD4+ and CD8+ T-cells and the innate immune process to protection from infections should be evaluated and then transferred to vaccine-induced primary immunity. Vaccines need to be produced efficiently and administered in a manner acceptable to the recipient. In the earliest vaccines, either living (attenuated versions of the pathogen or organisms capable of causing cross-reactive immunity but less virulent without inducing disease) or dead all organisms were used. All organisms have the advantage of being highly immunogenic and typically stimulate a response similar to that produced by natural infection. However, they can also produce the pathology caused by natural infection (17).

Vaccines can take a long time to make and distribute, and ensuring adequate supply worldwide is another challenge. One of the most challenging aspects of vaccine design is continuing to evaluate the effectiveness/efficacy of new vaccine formulations. The road to licensure involves understanding the biology and immunology of host-pathogen interactions. For this, immunological criteria, the nature of the vaccine formulation, its relationship with the antigens selected to maintain the specific immune response, the evaluation of immune responses, and testing in animal models and humans are aimed. Evaluating whether adjuvants are necessary to enhance the immune response to the vaccine antigen is a key decision. Likewise, the ability to develop a scalable, robust and repeatable formulation suitable for production is critical to a successful license. Facilities are required for the production and packaging of the vaccine. Proper storage of vaccines and monitoring at administration sites is critical because improper storage can render the vaccine to a non-immunogenic vaccine or potentially harmful (18).

Vaccine Development Stages

Combining the correct antigens and adjuvants to optimize the adaptive immune response is essential in the development of any new vaccine, and the production of vaccine antigens should be practically feasible (17). The time required for a vaccine to be produced and distributed varies by product and type of vaccine. Production can be relatively simple or more complex. Additional studies such as determination of viability and shelf life for live attenuated vaccines are required. From the moment the raw materials are supplied, it may take 10-26 months for vaccines to be ready for shipment. Thanks to technological developments, it is possible to accelerate these stages, but it is possible to return to the beginning after a mistake and extend the time. Continuous monitoring of efficacy and safety in vaccinated populations is essential to maintain confidence in vaccine programs (18). The development of any new vaccine includes the following stages (17).

Life cycle of the pathogen and epidemiology: Knowledge of pathogen structure, route of entry, interaction with cellular receptors, replication sites and disease-causing mechanisms are important to identify appropriate antigens for disease prevention. Demographic characteristics of the infection, specific risk groups, and age-specific infection rates determine which population will be vaccinated at what age.

Immune control and escape: Interactions between host and pathogen are investigated by determining the relative importance of antibodies, different types of T-cells and innate immunity, immune escape strategies during infection, and possible immune relationships of protection. This information guides the specific immune response required for protection, as well as the identification and selection of the antigen.

Antigen selection and vaccine formulation: The selected antigen can be formulated so that it remains suitably immunogenic and stable over time, elicits a potentially protective immune response, and is also ultimately amplified for commercial production.

Vaccine preclinical and clinical testing: The candidate vaccine must be tested for immunogenicity, safety and efficacy in preclinical and appropriately designed clinical trials (17). Studies of the phase until the vaccine is put on the market are given (Table 1) (18).

Vaccines are the most effective and economical way to prevent and control infectious diseases (19). Due to the human and economic effects of COVID-19, it has become necessary to evaluate new generation vaccine technology platforms and to accelerate the vaccine development studies against this pandemic (20). Rapid vaccine development is a global imperative to

prevent COVID-19. A higher level of community immunity can be achieved thanks to the pandemic ability of viruses to spread. However, this may happen with recurrent waves of infection in the next few years until an effective vaccine against SARS-CoV-2 is found or approximately 60-70% of people develop immunity. The consequences of recurrent outbreaks may result in unacceptably high mortality and serious economic losses, and ultimately necessitate major changes in human lifestyle. It is therefore imperative to ensure access to approved vaccines available for large-scale distribution over the next 1-2 years (16).

Since the world has not yet developed a vaccine or drug against COVID-19, it is trying to gain time and immunity by delaying the spread of the disease (21). While new outbreaks from newly emerging viruses are inevitable, scientists, epidemiologists and the healthcare industry continue to develop vaccines and antivirals using global viral surveillance programs and race new technologies to minimize and control the epidemic (1).

COVID-19 Current Vaccine Strategies

SARS-CoV-2 viral RNA encodes various proteins, including four structural proteins whose potential targets are Spike (S), Envelope (E), Membrane (M), and Nucleocapsid (N) (17) (Figure 2) (22). CoV S protein is the main envelope glycoprotein and is the main determinant of protective immunity. The S protein consists of two basic subunits, S1 and S2. S1 directs receptor binding while S2 is responsible for membrane fusion. Due to the high selective pressure and tropism determinants, the S protein is the most diverse region of CoV. This diversity poses a major challenge to the progress of vaccine development. Surface glycoproteins are the main target for vaccine development (1). The S protein is the outermost part of the virion that shows high glycosylation among the surface proteins of CoV on the envelope. In addition, it is an important structural protein that allows the virus to attach to the specific cell and enter the host cell (23). One of the main targets of CoV vaccines in humans is to elicit a strong humoral immune response against the S protein (1). It is stated that 12-18 months may be required for the development of a producible, safe and effective vaccine for COVID-19 (24). MERS-CoV-specific CD4+ and CD8+ T cells, learned from previous MERS-CoV infection, were detected in mononuclear blood cells of infected individuals. Therefore, the balance of T and B cell responses has often been considered the gold standard for preventing and resolving MERS-CoV and SARS-CoV infection (1). Hyperimmunoglobulin isolated from the sera of recovered patients who produce high antibody titers may provide passive immunity against SARS-CoV-2. It is stated that antibody-dependent therapy may represent the most effective, short-term therapeutic intervention, if S protein targeting, regulatory and safety requirements can be addressed (24). Since the S proteins between SARS-CoV and SARS-CoV-2 show only 76-78% sequence similarity, it reduces the likelihood that existing test vaccines and antivirals will protect against SARS-CoV-2 (1).

Rapid diagnosis, vaccines and therapeutics are extremely important interventions for COVID-19 pandemic management (25). Many countries, companies and institutions share their COVID-19 action programs and their developments in vaccine development against this virus with the world. Today, many of

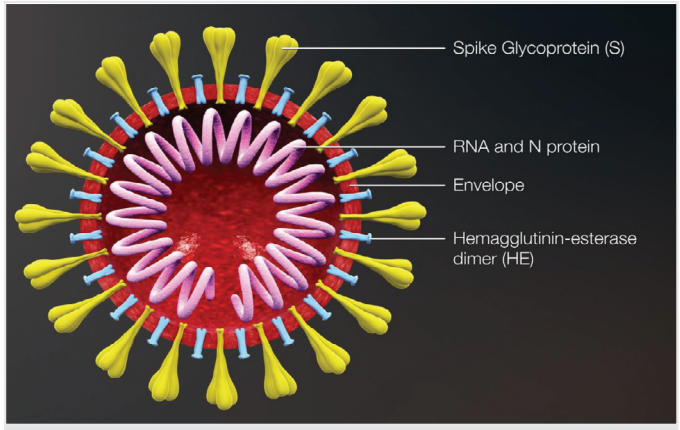

Figure 2. Coronavirus cross section

Table 1. Vaccine Phase Studies

Phase-1 studies	In this phase, the aim is to collect the initial safety and immunogenicity data of the vaccines and these individuals, typically containing <100 volunteers, are healthy individuals.
	These studies are also used for dose and sometimes program optimization.
	These studies typically involve 100 volunteers in the targeted patient population.
Phase-2 studies	In this phase, it is aimed to verify the dose amount, frequency and route of administration, to create immunogenicity data in the target population, to collect additional safety data and, if possible, to show "proof of concept" from the immunogenicity and/or efficacy endpoints.
	Phase-3 studies, known as registration, typically involve 1,000 volunteers in the target population.
	It frequently includes randomized control trials in which recipients are placed in actively vaccinated groups.
Phase-3 studies	These large sample sizes are used to adequately address the immune response, efficacy, and reactogenicity of the candidate vaccine.
	These studies could also examine the immunity and safety profile of the candidate vaccine when administered with other vaccines routinely administered to the target population.
Phase-4 studies	In parallel with Phase-3 clinical development, pilot-scale manufacturing progresses to full-scale production, including production of vaccine lots, and clinical testing to verify manufacturing consistency, and then Phase-4 is reached and the vaccine is released and licensed.

the vaccines studied against COVID-19 are in the design and preparation phase, but there are some vaccines whose effectiveness was evaluated in the first clinical studies and in animals (26).

COVID-19 Candidate Vaccine Production Studies

Since there is no coronavirus vaccine in the market and there is not yet a large-scale production capacity for these vaccines, these processes and capacities must first be established. Doing this for the first time can be tedious and time consuming (27,28). But the biggest challenge of vaccine and antiviral development is the elusive nature of viruses that often arise from populations of highly heterogeneous virus strains circulating in animal reservoirs. The evolutionary dynamics of RNA viruses are complex and their high mutation rates, fast replication kinetics, and large population sizes pose major challenges for traditional population genetics (21). There is no approved HCoV vaccine so far. The development of vaccines that can be applied to humans can take years, especially since their safety needs to be tested in detail and superior new technological applications are required for serial production (29). The Coalition for Epidemic Preparedness Innovations (CEPI) has funded a large number of innovative researchers in the field, and these researchers are seriously working to develop the SARS-CoV-2 vaccine. However, none of these companies and institutions have an established drug development pipeline and the capacity to produce the required number of doses to bring such a vaccine to late-stage clinical trials that allow for licensing. An mRNA-based vaccine that expresses the target antigen *in vivo* after injection of mRNA encapsulated in lipid nanoparticles developed jointly by Moderna and the Vaccine Research Center of the National Institutes of Health is currently the furthest step. CureVac is also working on a similar vaccine, even though it is still in the preclinical stage. Additional approaches in the pre-clinical phase include recombinant-protein-based vaccines (ExpreS2ion, iBio, Novavax, Baylor College of Medicine and Sichuan Clover Biopharmaceuticals), viral-vector-based vaccines (Vaxart, Geovax, Oxford University and Cansino Biologics), DNA vaccines (Inovio and Applied DNA Sciences), live attenuated vaccines (Serum Institute and Codagenix) and inactivated virus vaccines (Table 2) (27). Inactivated vaccines are an important traditional vaccine type that can be easily produced and developed quickly. In this approach, SARS-CoV-2 virions can be chemically and/or physically inactivated to elicit neutralizing antibodies. In studies of SARS-CoV and MERS-CoV, neutralizing antibodies have been successfully and robustly induced by an inactivated vaccine in all animal experiments, but still need testing as there are concerns about the development of antibody-related viral infection and other safety issues. Other alternative vaccine approaches requiring further research and testing in animals, including live attenuated vaccines, subunit vaccines and vectored vaccines, should also gain momentum (27,28).

All these platforms have advantages and disadvantages, and it is impossible to predict which strategy will be faster or more successful. Johnson & Johnson (J&J) and Sanofi have recently participated in SARS-CoV-2 vaccine development. While J&J uses an experimental adenovirus vector platform that has not yet resulted in a licensed vaccine, the Sanofi vaccine is planned to be made using a process similar to that used for the approved Flublok recombinant influenza virus vaccine, but they estimate that it will result in human readiness within months (27).

Table 2. Overview of Vaccine Production Platforms and Technologies for SARS-CoV-2.

SARS-CoV-2 Vaccines: Various Approaches

All vaccines aim to expose the body to an antigen that does not cause the disease, but if a person becomes infected it causes an immune response that can block or kill the virus. There are at least eight types of vaccines being tested against CoV. These types of vaccines consist of different viruses or viral parts, such as genetically modified measles and adenovirus (30).

Viral vaccines: These vaccines are developed using the virus itself, in a weakened or inactivated form. There are at least seven teams involved in developing virus vaccines. Many of the current vaccines, such as those against measles and polio, are made this way, but they require extensive safety testing. Looking at examples from around the world about virus vaccines; Sinovac Biotech in Beijing has begun testing an inactive version of SARS-CoV-2 in humans (30). Again, researchers at the University of Hong Kong continue to work on vaccine development by weakening the influenza virus in virus vaccine studies. molecular clamp technology. This polypeptide retains the twisted S protein so that the body's immune system recognizes it before the virus is activated (31).

Viral vector vaccines: There are about 25 groups reporting that they are working on viral vector vaccines. A virus such as measles or adenovirus was genetically engineered to produce CoV proteins in the body. There are two types of these viruses: those that can still reproduce inside cells, and those that cannot cause disease because key genes are disabled (30). Over the past century, outbreaks have been successfully controlled thanks to vaccines (cholera, typhoid, polio, measles, plague, tetanus) developed using various technologies, mainly with classical pathogen inactivation or attenuation (32). Companies (Sanofi and GlaxoSmithKline) have collaborated and have been continuing their work rapidly for recombinant vaccines that use genetic technology for the treatment of COVID-19 (33). Viral vector vaccines are vaccines produced using recombinant DNA technology, and this vaccine type studies continue intensively. It can be classified into two main categories: DNA vaccines and recombinant (protein subunit) vaccines. DNA vaccines consist of synthetic DNA containing the gene encoding the disease-agent protein. Recombinant (protein subunit) vaccines are vaccines that contain a small part of the microorganism rather than the whole one. Hepatitis B vaccine is an example (34). When viral vector vaccine studies are analyzed; Novavax uses recombinant protein nanoparticle technology that presents viral S protein-derived antigen in vaccine development. Pasteur Institute (France), on the other hand, continues to work against the SARS-CoV-2 virus by adapting the vectorial measles virus. In addition, Oxford University (England) tested the ChAdOx1 nCoV-19 vector vaccine funded by CEPI. This vaccine was

tested on animals at the beginning of March and Phase 1 and Phase 2 trials were started in 510 volunteers at the end of March. CanSino Biologics (China) conducted a Phase 1 safety trial in Wuhan on 18 March in 108 volunteers to test the recombinant adenovirus vaccine candidate Ad5-nCoV, and a Phase 2 trial on 500 volunteers on April 12 (31). The Dokuz Eylul University in Turkey (İzmir), spike protein recombinant vaccine development continues to work against and ACE-2 receptor (35).

Nucleic acid vaccines: These vaccines aim to use genetic instructions (in the form of DNA or RNA) for a coronavirus protein that causes an immune response. There are at least 20 teams working on nucleic acid vaccines. Most of these vaccines encode the spike protein of the virus, and then the nucleic acid that makes copies of the virus protein is introduced into human cells (30). There are many major biotechnologies that have advanced nucleic acid vaccine platforms for COVID-19. For example, INOVIO Pharmaceuticals is developing a DNA vaccine, while others such as Moderna Therapeutics and CureVac are researching RNA vaccine platforms (36-38). The candidate vaccine from Moderna is an RNA vaccine developed using technology that is faster, cheaper, and easier to scale than traditional vaccine methods. The vaccine promises to be more reliable as it is not produced by weakening living viruses, but based on a synthetic RNA molecule encoding a single viral protein. CureVac is also working against the mRNA-based SARS-CoV-2 virus. In addition, vaccination studies are accelerating to allow human trials in parallel with animal trials due to the pandemic. Based on the positive results of the INO-4800 DNA vaccine developed by Inovio Pharmaceuticals (USA), it was allowed to be tested in the Phase 1 clinical phase in 40 volunteers and the first dose in humans was administered on April 6 (31). In Turkey, Ankara University (Ankara) carries out DNA and peptide vaccine development studies against COVID-19. Again, the first step of DNA vaccination studies carried out by Ege University (İzmir) has been completed and the second phase has been started (35).

Table 2. Overview of Vaccine Production Platforms and Technologies for SARS-CoV-2

Platform	Target	Available, Licensed Human Vaccines Using the Same Platform	Advantages	Disadvantages
RNA vaccines	S protein	No	No infectious virus needs to be handled, vaccines are typically immunogenic, rapid production possible.	Safety issues with reactogenicity have been reported.
DNA vaccines	S protein	No	No infectious virus needs to be handled, easy scale up, low production costs, high heat stability, tested in humans for SARS-CoV-1, rapid production possible.	Vaccine needs specific delivery devices to reach good immunogenicity.
Recombinant protein vaccines	S proteini	Yes for baculovirus influenza, HPV) and yeast expression (HBV, HPV).	The infectious virus does not need to be addressed, vaccines are typically immunogenic, rapid production is possible.	Global production capacity may be limited. Antigen and/or epitope integrity needs to be verified. Their efficiency must be high enough.
Viral vector-based vaccines	S proteini	Yes for VSV (Ervebo), but not for other viral vector vaccines.	No infectious virus needs to be addressed, excellent preclinical and clinical data for many new viruses, including MERS-CoV.	Vector immunity can adversely affect vaccine efficacy (depending on the vector chosen).
Live attenuated vaccines	Whole virion	Yes	Simple operation used for various licensed human vaccines, existing infrastructure can be used.	Creating infectious clones for attenuated CoV vaccine seeds takes time due to the large genome size. Security tests must be comprehensive.
Inactivated vaccines	Whole virion	Yes	Simple procedure used for various licensed human vaccines, existing infrastructure can be used, tested for SARS-CoV-1 in humans, adjuvants can be used to increase immunogenicity.	Large amounts of infectious virus need to be addressed (can be mitigated using an attenuated seed virus). Antigen and/or epitope integrity needs to be verified.

HBV: Hepatitis B Virus, HPV: Human papilloma virüs, MERS-CoV: Middle east respiratory syndrome coronavirus, SARS-CoV: Severe acute respiratory syndrome coronavirus

Conclusion and Recommendatıons

Three pandemics caused by SARS, MERS, and SARS-CoV-2 are believed to have started as a result of bat coronaviruses that crossed the species barrier. Therefore, other outbreaks are likely to occur in the future due to the unlimited supply of coronavirus available in the bat population. Prevention is always the best treatment, and continuous viral surveillance of wild animals is extremely important for potentially emerging CoVs. Clinical trials begin with small safety studies in animals and humans, followed by much larger studies to determine whether a vaccine induces an immune response. As a result of all these, it may take many years for the vaccine to emerge, so fast, reliable and easy-to-use viral test kits should be developed on the research side. The continuous development of vaccines and antivirals will provide an assurance of fighting and controlling emerging CoV diseases.

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Authorship Contributions

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References

1. Tse LV, Meganck RM, Graham RL, Baric RS. The Current and Future State of Vaccines, Antivirals and Gene Therapies Against Emerging Coronaviruses. Front Microbiol 2020;11:658.
2. World Health Organization Press Conference 2020 The World Health Organization (WHO) Has Officially Named the Disease Caused by the Novel Coronavirus as COVID-19. Last Accessed Date:12.05.2020. Available from: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200312-sitrep-52-covid-19.pdf?sfvrsn=e2bfc9c0_4.
3. Cascella M, Rajnik M, Cuomo A, Dulebohn S, Napolli R. Features, Evaluation and Treatment Coronavirus (COVID-19); Stat Pearls Publishing: Treasure Island, FL, USA: 2020.
4. Fehr AR, Perlman S. Coronaviruses: An Overview of Their Replication and Pathogenesis. Methods in Molecular Biology (Clifton, N.J.) 2015;1282:1-23.
5. Raj VS, Osterhaus AD, Fouchier RA, Haagmans BL. MERS: Emergence of a Novel Human Coronavirus. Curr Opin Virol 2014;5:58-62.
6. Jin Y, Yang H, Ji W, Wu W, Chen S, Zhang W, Duan G. Virology, Epidemiology, Pathogenesis, and Control of COVID-19. Viruses 2020;12:372.
7. Li H, Liu SM, Yu XH, et al. Coronavirus Disease 2019 (COVID-19): Current Status and Future Perspective. Int J Antimicrob Agents 2020;55:105951.
8. Baig AM, Khaleeq A, Ali U, Syeda H. Evidence of the COVID-19 Virus Targeting the CNS: Tissue Distribution, Host-Virus Interaction, and Proposed Neurotropic Mechanisms. ACS Chemical Neuroscience 2020;11:995-8.
9. Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. Into the eye of the cytokine storm. Microbiol Mol Biol Rev 2012;76:16-32.
10. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. Lancet 2020;395:507-13.
11. Zhou JH, Wang YN, Chang QY, Ma P, Hu Y, Cao X. Type III Interferons in Viral Infection and Antiviral Immunity. Cell Physiol Biochem 2018;51:173-85.
12. Rouse BT, Lukacher AE. Some unmet challenges in the immunology of viral infections. Discovery Med 2010;10:363-70.
13. Zimmermann P, Curtis N. Factors That Influence the Immune Response to Vaccination. Clin Microbiol Rev 2019;32:e00084-18.
14. Broadbent AJ, Boonnak K, Subbarao K. Respiratory Virus Vaccine. Mucosal Immunology 2015:1129-70.
15. Gül B, Dikmen BY. Aşı Adjuvanları ve İstenmeyen Etkileri. Veteriner Farmakoloji ve Toksikoloji Derneği Bülteni 2019;10:91-105.
16. Graham BS. Rapid COVID-19 Vaccine Development. Science 2020;368:945-6.
17. Cunningham AL, Garçon N, Leo O, Friedland LR, Strugnell R, Laupèze B, et al. Vaccine development: from concept to early clinical Testing. Vaccine 2016;34:6655-64.
18. Preiss S, Garçon N, Cunningham AL, Strugnell R, Friedland LR. Vaccine provision: delivering sustained & widespread use. Vaccine 2016;34:6665-71.
19. Remy V, Largeron N, Quilici S, Carroll S. The economic value of vaccination: why prevention is wealth. Value Health 2015;17:450.
20. Le TT, Andreadakis Z, Kumar A, Roman RG, Tollefsen S, Saville M. et al. The COVID-19 Vaccine Development Landscape. Nature Reviews Drug Discovery 2020;19:305-6.
21. Lauring AS, Andino R. Quasispecies theory and the behavior of RNA viruses. PLoS Pathogens 2010;6:1-8.
22. Why the Coronavirüs and Most Other Viruses Have No Cure. Last Accessed Date: 12.06. 2020. Available from: https://www.inquirer.com/health/coronavirus/coronavirus-covid19-antiviral-cure-antibiotic-20200318.html
23. Guo YR, Cao QD, Hong ZS. The Origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak–an update on the Status. Mil Med Res 2020;7:1-10.
24. Osman EEA, Toogood PL, Neamati N. COVID-19: Living through another pandemic. ACS Infect Dis 2020;6:1548-52.
25. Pang J, Wang MX, Ang I, Tan SHX, Lewis RF, Chen JIP, et al. Potential rapid diagnostics, vaccine and therapeutics for 2019 novel coronavirus (2019-nCoV): A systematic review. J Clin Med 2020;9:623.
26. Zhang J, Zeng H, Gu J, Li H, Zheng L, Zou Q. Progress and prospects on vaccine development against SARS-CoV-2. Vaccines 2020;8:153.
27. Amanat F, Krammer F. SARS-CoV-2 vaccines: status report. Immunity 2020;52:583-9.

28. Yuen KS, Ye ZW, Fung SY, Chan CP, Jin DY. SARS-CoV-2 and COVID-19: The most ımportant research questions. Cell Biosci 2020;10:1-5.

29. Sağlık Bakanlığı 2020 COVID-19 Yeni Koronavirüs Hastalığı. Last Accessed Date: 12.05.2020. Available from: https://covid19bilgi.saglik.gov.tr/tr/sss/halka-yonelik.html

30. Callaway E. The Race for coronavirus vaccines: a graphical guide. Nature 2020;580:576-7.

31. Editorial. Race for a COVID-19 Vaccine. Science Direct 2020;55:102817.

32. Kim YC, Dema B, Reyes-Sandoval A. COVID-19 Vaccines: Breaking Record Times to First-in-Human Trials. npj Vaccines 2020;5:1-3.

33. GSK 2020 GSK actions to support the global response to COVID-19. Last Accessed Date: 12.05.2020. Available from: https://www.gsk.com/en-gb/media/press-releases/sanofi-and-gsk-to-join-forces-in-unprecedented-vaccine-collaboration-to-fight-covid-19/

34. Aytar M, Başbülbül G. Rekombinant Aşılar. Elektronik Mikrobiyoloji Derg 2019;17:1-10.

35. COVID-19 Türkiye Web Portalı 2020. Last Accessed Date: 12.05.2020. Available from: https://covid19.tubitak.gov.tr/duyurular/covid-19-turkiye-platformu-asi-ve-ilac-gelistirme

36. INOVIO 2020 Urgently Focused on Developıng Covid-19 Vaccine Because the World Can't Wait. Last Accessed Date: 12.05.2020. Available from: https://www.inovio.com/our-focus-serving-patients/covid-19/

37. Smith J. 2020 Curevac Bids to Develop First mRNA Coronavirus Vaccine. Available from: https://www.labiotech.eu/medical/curevac-coronavirus-outbreak-cepi/

38. Park A. Inside the company that's hot-wiring vaccine research in the race toc ombat the coronavirus 2020 Time. Last Accessed Date: 12.05.2020. Available from: https://time.com/5775784/coronavirus-vaccine-research/ Erişim Tarihi: 12.05.2020.

Review

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SARS CoV-2 Pathogenesis and Immune Response

SARS-CoV-2 Patogenezi ve İmmün Yanıt

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ABSTRACT

The agent responsible for the epidemic that first appeared in Wuhan, China, in December 2019 was detected to be the new coronavirus (2019-nCoV). Later, the virus that caused respiratory tract infection was discovered to be a member of the beta-coronavirus family, it was named as severe acute respiratory syndrome-CoV2 (SARS-CoV 2), and the disease it caused was called CoV infection disease-19 (COVID-19). The epidemic started in China, spread rapidly first to East Asian countries, then Europe and America, affected the whole world, and was declared a pandemic by the World Health Organization. This review presents an overview of SARS-CoV2 and aims to examine its intracellular pathogenesis and host immune responses.

Keywords: Coronavirus, COVID-19, pathogenesis, immunology, SARS-CoV-2

ÖZ

İlk kez Çin'in Wuhan kentinde Aralık 2019'da ortaya çıkan salgından sorumlu olan etkenin yeni koronavirüs (2019-nCoV) olduğu saptanmıştır. Daha sonra, solunum yolu enfeksiyonuna yol açan bu virüsün beta-CoV ailesine ait olduğu belirlenip, şiddetli akut solunum yolu enfeksiyonu-koronavirüs-2 (SARS-CoV-2) olarak adlandırılmış ve oluşturduğu hastalığa da koronavirüs enfeksiyon hastalığı-19 (COVİD-19) denilmiştir. Çin'de başlayan salgın hızla Doğu Asya ülkelerine sonrasında Avrupa ve Amerika'ya yayılarak, tüm dünyayı etkisi altına almış ve Dünya Sağlık Örgütü tarafından pandemi olarak ilan edilmiştir. Bu derleme, bu virüsle ilgili genel bir bakış açısı sunmakla birlikte, hücre içindeki patogenezini ve konak immün yanıtlarını incelemeyi amaçlamıştır.

Anahtar Sözcükler: Koronavirüs, COVİD-19, patogenez, immünoloji, SARS-CoV-2

Introduction

Coronaviruses (CoVs) are enveloped, positive-strand RNA viruses with large genomes (26-32 kb) (1). Some of the enveloped CoVs belonging to the large Coronaviridae subfamily that infect birds and mammals were known to cause self-limited infections in humans, mostly affecting the respiratory system. However, CoVs became important pathogens again as they caused serious outbreaks in the last 20 years, such as the severe acute respiratory syndrome CoV (SARS-CoV) in 2002 and the Middle East respiratory syndrome CoV (MERS-CoV) in 2012. In December 2019, the source of the epidemic, which was believed to be associated with seafood and wet animal market in Wuhan, China, was understood to be a new type of coronavirus, and on February 11, the World Health Organization (WHO) named the disease caused by the virus as COVID-19 (2). Molecular studies revealed that the new coronavirus belonged to the beta coronavirus group, and its sequence was 88% similar to two bat-origin SARS-like CoVs. (3). While initially it was named as 2019-nCoV, the International Commission on Virus Classification announced the name of this novel coronavirus as SARS-CoV-2. Due to a rapid spread of SARS-CoV-2 to many countries of the world and the increased mortality, WHO declared the COVID-19 outbreak

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as a "pandemic" on March 11, 2020 (4). While most of the patients infected with SARS-CoV-2 had a mild clinical course, approximately 5% of the patients had severe lung damage or even multiple organ failure. Although it varies by country, the average death rate has been reported as 4%. (5). As of the end of June, 216 countries all over the world were affected by the epidemic, 10,021,401 people were confirmed to be infected with SARS-CoV-2, and 499,913 people died (6).

SARS-CoV-2 Replication and Pathogenesis

There are four main structural proteins in the structure of SARS-CoV-2: spike (S), envelope (E), membrane (M), and nucleocapsid (N) glycoproteins, as well as various accessory proteins (7) (Figure 1). Although the spike protein is located on the outer surface of the virus, it consists of two subunits. The S1 subunit plays a role in the binding of the virus to the receptor on the host cell surface, while the S2 subunit mediates cell fusion (8,9). S protein binds to the angiotensin-converting enzyme-2 (ACE-2) receptor of the host cell, and this complex is subjected to a proteolytic process by the host type II transmembrane serine protease (TMPRSS2) enzyme, and the virus enters the cell (10,11). The virus needs both ACE-2 and TMPRSS2 to enter the cell (12). A single-stranded positive-polar RNA with 5 "cap and 3" polyA tail enters the cell. There are about 14 orf's (open reading frames) encoding structural and non-structural proteins in this virus genome (13). First, this genomic RNA is broken down into small pieces by viral proteinases, and non-structural viral replicase polyproteins (pp1a and pp1b) are produced due to the translation of *orf1* and *orf2* genes. Polymerase enzyme synthesizes subgenomic mRNAs and acts as a template for synthesized negative polarity sgRNA mRNA. Translation of viral proteins also takes place from mRNAs. Finally, viral proteins and RNA genome combine within virions in the endoplasmic reticulum and Golgi body and are released into the environment as a result of the fusion of vesicles containing viral particles outside the cell with the cell plasma membrane (7) (Figure 2). At this stage, M protein plays an important role in stabilizing the N protein-RNA complex in the virion, keeping it together, and leaving the cell (13).

It is known that SARS-CoV-2 is transmitted by droplets, just like other human CoVs, and causes primary infection by binding to ACE-2 receptors that are abundant in lower respiratory tract epithelium. The mean incubation period was reported to be 4-5 days before the onset of symptoms, and 97.5% of symptomatic patients have symptoms within an average of 11.5 days (14). Typical manifestations of COVID-19 include fever and dry cough, although symptoms also include shortness of breath, muscle and/or joint pain, headache, diarrhea, nausea (5,15,16). Acute respiratory distress syndrome (ARDS) can be seen 8-9 days after the onset of symptoms in patients with severe COVID-19 (15,17). Biopsy and autopsy examinations of some COVID-19 patients have shown damage to the alveoli (18). Although the pathophysiology and virulence characteristics of the virus have not been clearly revealed yet, they are possibly related to the structural and non-structural proteins of the virus. Although many studies found ACE-2 receptors, which the virus binds in the small intestine, liver, kidney, and the central nervous system, no study has found viral particles in these organs.

SARS-CoV-2 Immunopathology

Host immune system develops various antiviral defense mechanisms against virus infections. Pattern recognition receptors (PRR) in host cells, especially Toll-like receptors (TLR) located in the cell, recognize RNA products formed during viral replication. As a result of this recognition, the immune system is activated by the secretion of type I interferon (type I IFN) from virus-infected cells (19). IFN suppresses protein synthesis in the infected cell by stimulating the RNA-dependent protein kinase enzyme, preventing viral replication and protecting uninfected cells against infection (20). Antigenic structures of pathogens are presented to CD8 T lymphocytes by antigen-presenting cells via MHC-I, and cytotoxic T-lymphocytes are activated with stimulation of cytokines interleukin [(IL)-12, IFN-α/β)]. In addition, some dendritic cells can present the processed antigen to both CD4 and CD8 T-lymphocytes by phagocytosing the virus-infected cell (21).

The specific role of humoral and cellular immunity or innate immunity in relation to SARS-CoV-2 is not known, but it probably induces a similar immune response to SARS-CoV and MERS-CoV. RNA viruses are generally recognized by TLR-3

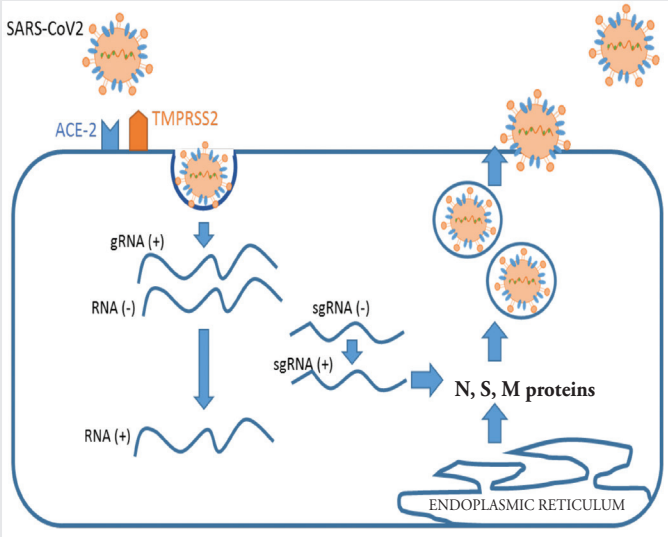

Figure 2. SARS-CoV-2 intracellular cycle

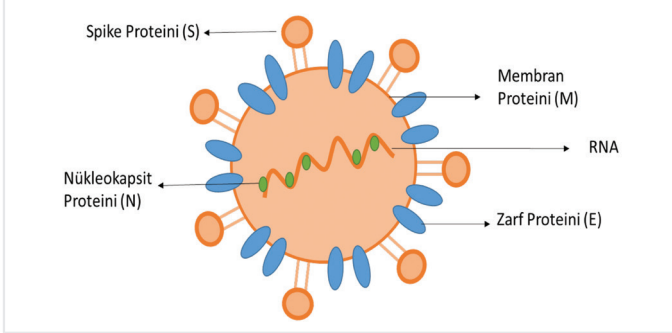

Figure 1. SARS-CoV-2 virus structure

and TLR-7 endosomal PRRs, and immune system activation begins (22). The production of proinflammatory cytokines and chemokines (IL-6, IP-10, macrophage inflammatory protein 1α (MIP1α), MIP1β, and MCP1) is triggered by stimulation from neighboring epithelial cells, endothelial cells, and alveolar macrophages (23). These cytokines and chemokines attract monocytes, macrophages, and T cells to the infection site, and the immune response is enhanced by IFN -γ production by the T cells.

Increased release of proinflammatory cytokines causes a cytokine storm in COVID-19 patients. Also, increased expression of proinflammatory cytokines during such cytokine storms causes depletion of lymphocytes (24). Overproduction of proinflammatory cytokines induced by the virus results in cytokine storm, defined as macrophage activation syndrome (MAS) or secondary hemophagocytic lymphohistiocytosis (25-27). Severe cases infected with SARS-CoV-2 can be characterized by a cytokine storm that progresses to ARDS; also, high levels of these cytokines in the circulation can lead to shock and tissue damage in the heart, liver, and kidney, as well as respiratory failure or multiple organ failure (28). This high proinflammatory cytokine (especially TNF-a, IL-6, IL-1) level seen in patients with severe COVID-19 is similar to SARS-CoV and MERS-CoV (29,30).

Depending on flowcytometric analysis of peripheral blood in patients infected with SARS-CoV-2 overactivation of T cells with high cytotoxicity of Th17 and CD8 T cells were and immune damage in these patients was emphasized (18). Previous research demonstrated increased levels of IL-1β, IL-6, IL-2, IL-8, IL-17, G-CSF, GM-CSF, IP10, MCP1, MIP1α, and TNF-α levels in sera of COVID-19 patients suggesting a clinical picture of cytokine storm (15,18,31,32). In addition, COVID-19-associated pneumonia was associated with strong interferon suppression with lymphopenia as part of lung injury, ARDS, and virus-induced immunosuppression (33). Along with low lymphocyte counts, these patients also have a decrease in CD4 and CD8 T cell counts (31). It is thought that SARS-CoV2 may escape the antiviral mechanism (34).

Conclusion

Although SARS-CoV-2 is in the coronavirus family, its pathophysiology has not been clearly understood yet. Studies on SARS-CoV2, which has become a pandemic in a short time in the world, often refer to the diagnosis and treatment options of the agent, but also include case reports. The number of patients in studies to explain the immune response, especially in COVID-19 patients, is very low. In order to fully and adequately explain the mechanism of immunopathogenesis, larger samples should be used. However, conducting research on virulence properties, intracellular mechanisms, immune stimuli, and escape mechanisms from the host immune response of SARS-CoV2 will bring a different perspective to approaches to the treatment of COVID-19 caused by the pathogen. Unfortunately, zoonotic viruses (Ebola, Zika, Dengue virus, West Nile Fever, MERS-CoV, SARS-CoV), including SARS-CoV-2, are serious threats today in a globalized world. Global warming may induce mutations in viruses by changing the habitat of viruses and their vectors. As a result of increased human mobility, the rapid spread of viruses through infected individuals has been seen again in the COVID-19 pandemic. In addition, it is noteworthy that bats, which are the only flying mammal and contain approximately 4,000 virus species, of which approximately 1,500 of these viruses are coronavirus, should be examined as a possible risk factor. Genomic research about mutations of viruses carrying potential risks is important to protect public health.

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References

1. Su S, Wong G, Shi W, Liu J, Lai ACK, Zhou J, et al. Epidemiology, Genetic recombination, and pathogenesis of coronaviruses. Trends Microbiol 2016;24:490-502.
2. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 2020;579:270-3.
3. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 2020;395:565-74.
4. World Health Organization. (2020). https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-mission-briefing-on-covid-19---12-march-2020.
5. Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020 Feb 28. doi: 10.1056/NEJMoa2002032. [Epub ahead of print]
6. World Health Organization, Coronavirus disease (COVID-19) pandemic. (2020). Last Accessed Date: 30.06.2020 Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019.
7. Shereen MA, Khan S, Kazmi A, Bashir N, Siddique R. COVID-19 infection: origin, transmission, and characteristics of human coronaviruses. J Adv Res 2020;24:91-8.
8. Fehr AR, Perlman S. Coronaviruses: an overview of their replication and pathogenesis. New York: Springer; 2015:1-23.
9. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020;181:281-92.
10. Glowacka I, Bertram S, Muller MA, Allen P, Soilleux E, Pfefferle S, et al. Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. J Virol 2011;85:4122-34.

11. Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O, Pohlmann S. TMPRSS2 and ADAM17 Cleave ACE2 Differentially and Only Proteolysis by TMPRSS2 Augments Entry Driven by the Severe Acute Respiratory Syndrome Coronavirus Spike Protein. J Virol 2014;88:1293-307.
12. Shulla A, Heald-Sargent T, Subramanya G, Zhao J, Perlman S, Gallagher TA. Transmembrane Serine Protease Is Linked to the Severe Acute Respiratory Syndrome Coronavirus Receptor and Activates Virus Entry J Virol 2011;85:873-82.
13. Astuti I, Ysrafil. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. Diabetes Metab Syndr 2020;14:407-12.
14. Lauer SA, Grantz KH, Bi Q, Jones FK, Zheng Q, Meredith H, et al. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. Ann Intern Med 2020;172:577-82.
15. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506.
16. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. Clinical and immunologic features of severe and moderate coronavirus disease 2019. J Clin Invest 2020;130:2620-9.
17. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020;323:1061-9.
18. Xu Z, Shi L, Wang Y. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet 2020;8:420-2.
19. Abbas AK, Lichtman AH, Pillai S. Basic Immunology: Functions and Disorders of the Immune System. 4th ed. Philadelphia: Elsevier Saunders; 2014.
20. Doan T, Melvold, Viselli S, Waltwnbaugh C. Lippincott's illustrated reviews serisinden: İmmünoloji. Ed: Deniz G, Erten G, Camcıoğlu Y. Nobel Tıp Kitabevleri, 2. Basım, 2017.
21. Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology. 8th ed. Philadelphia: Elsevier Saunders; 2015.
22. Felsenstein S, Herbert JA, McNamara PS, Hedrich CM. COVID-19: Immunology and treatment options. Clin Immunol 2020;215:108448.
23. Tay ZM, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: immunity, inflammation and intervention. Nature Rev Immunol 2020;20:363-74.
24. Cron RQ, Chatham WW. The rheumatologist's role in Covid-19. J Rheumatol 2020;47:639-42.
25. George MR. Hemophagocytic lymphohistiocytosis: review of etiologies and management. J Blood Med 2014;5:69-86.
26. Ramos-Casals M, Brito-Zeron P, Lopez-Guillermo A, Khamashta MA, Bosch X. Adult haemophagocytic syndrome. Lancet (London, England) 2014;383:1503-16.
27. McGonagle D, Sharif K, O'Regan A, Bridgewood C. Interleukin-6 use in COVID-19 pneumonia related macrophage activation syndrome. Autoimmun Rev 2020;19:102537.
28. Cao X. COVID-19: immunopathology and its implications for therapy. Nat Rev Immunol 2020;20:269-70.
29. Wong CK, Lam CW, Wu AKL, Ip WK, Lee NLS, Chan HIS, et al. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. Clin Exp Immunol 2004;136:95-103.
30. Min CK, Cheon S, Ha NY, Sohn KM, Kim Y, Aigerim A, et al. Comparative and kinetic analysis of viral shedding and immunological responses in MERS patients representing a broad spectrum of disease severity. Sci Rep 2016;6:25359
31. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clin Infect Dis 2020;71:762-8.
32. Tan M, Liu Y, Zhou L, Deng X, Li F, Liang K, et al. Immunopathological characteristics of coronavirus disease 2019 cases in Guangzhou, China. Immunology 2020;160:261-8.
33. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The role of cytokines including interleukin-6 in COVID-19 induced pneumonia and macrophage activation syndrome-like disease. Autoimmun Rev 2020;19:102537.
34. Yi Y, Lagniton PNP, Ye S, Li E, Xu RH. COVID-19: what has been learned and to be learned about the novel coronavirus disease. Int J Biol Sci 2020;16:1753-66.