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REVIEW ARTICLE

Pharmacological agents under investigation in the treatment of coronavirus disease 2019 and the importance of melatonin

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is a life-threatening infectious respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). COVID-19 pandemic causing morbidities and even deaths worldwide revealed that there is urgent need to find pharmacological agents or vaccines. Although there are a lot of agents under investigation, there is no approved agent for the prevention or treatment of the COVID-19 yet. Treatment of patients remains mainly supportive as well as compassionate use of the agents under investigation. It is well established that excessive inflammatory and immune response and oxidative injury play a critical role in the pathogenesis of COVID-19. In this review, we aimed to update knowledge about pathogenesis, clinical features, and pharmacological treatment of COVID-19 and review the potential beneficial effects of ancient antioxidant, anti-inflammatory, and immunomodulatory molecule melatonin for prevention and treatment of COVID-19.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first seen as cases of pneumonia of unknown cause in December 2019 in Wuhan, China. Unfortunately, it spread all over the world by human-to-human transmission and became a pandemic [1,2]. According to the situation report published by the World Health Organization (WHO), as of June 28, 2020, 495 760 people died from COVID-19 and there are 9 843 073 confirmed cases worldwide [3].

COVID-19 is a respiratory disease caused by SARS-CoV-2. Common symptoms of the COVID-19 are fever, dry cough, and fatigue. The following symptoms may also be seen: shortness of breath, aches and pains, sore throat, diarrhea, nausea, or a runny nose. Although COVID-19 causes mild to moderate symptoms in most infected individuals, there is a higher risk of developing severe disease and death in people with comorbid disease or over 60 years of age [4]. In cases developing severe disease, it was reported to cause acute lung injury (ALI)/acute respiratory distress syndrome (ARDS), respiratory failure, heart failure, sepsis, and sudden cardiac arrest within a few days [5]. It is well known that ARDS and multiple organ failure if they are not treated enough they may lead to death [6].

Despite all efforts, there is no approved vaccine or pharmacological agent for the prevention or treatment of the COVID-19 yet. Treatment of patients remains mainly supportive as well as compassionate or off-label use of the some unapproved agents. It is well known that inappropriate inflammatory and immune response and oxidative damage play an important role in the pathogenesis of COVID-19 [7,8].

Melatonin, an indolamine molecule, is mainly produced by pinealocytes in the pineal gland in humans. As a pleiotropic molecule, melatonin exerts substantial anti-inflammatory, antioxidant, and immunomodulatory properties [9].

In this review, we aimed to update knowledge about pharmacological treatment of COVID-19 and review

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the potential beneficial effects of melatonin for COVID-19 treatment, thus contributing to current scientific literature.

PATHOGENESIS OF COVID-19

Coronaviruses (CoVs) are belonged to ribonucleic acid (RNA) virus family mainly cause respiratory tract infections in mammals, including humans. Three highly pathogenic human CoVs (HCoVs), including SARS-CoV, Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2, infecting the lower respiratory tract and causing severe pneumonia even leading to fatal ALI and ARDS, have caused to outbreaks in past two decades [1,5].

The SARS-CoV-2 is known to have four viral structural proteins including spike (S) protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein. Translation of replicase/transcriptase and viral structural proteins occurs through viral genome acting as messenger RNA (mRNA). The replicase/transcriptase genes consist of open reading frame (ORF) 1a and ORF1b. The viral genome consists of the ORFs encoding replicase/transcriptase and the genes encoding viral structural proteins [6].

The SARS-CoV-2 is believed to enter the body via settling on the mainly nasal mucosa through droplets and contacts, then reach the lungs through respiratory system. The SARS-CoV-2 may infect the targets expressing angiotensin-converting enzyme 2 (ACE2) including the lung, heart, renal, and gastrointestinal system when viremia, originating from the lung, occurs [10]. Like the SARS-CoV, ACE2 was reported to be SARS-CoV-2 receptor for host cell entry with its S protein. S1 subunit of S protein is necessary for attachment whereas S2 subunit is necessary for viral fusion and cell entry. Recently, it was shown that transmembrane serine protease 2 (TMPRSS2), cleaves the S protein from S1/S2 and the S2' site for allowing viral fusion, primes S2 subunit for entry, and a serine protease inhibitor camostat mesylate hinders SARS-CoV-2 infection of lung cells [11,12]. The S protein of SARS-CoV-2 binds ACE2 with higher affinity than SARS-CoV [1].

The results of the clinical and preclinical studies have shown that inflammatory processes including release of cytokines and chemokines play an important role in the pathogenesis of infections caused by HCoVs. In *in vitro* studies, SARS-CoV and MERS-CoV infections were shown to associated with high levels of pro-inflammatory cytokines (interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)) and chemokines (C-C motif chemokine ligand (CCL)-2, CCL-3, and CCL-5); however, low levels of the antiviral factors interferons (IFNs) released by respiratory epithelial cells, dendritic cells (DCs), and macrophages. Elevated levels of cytokines and chemokines cause to neutrophil and monocyte infiltration in the lung, during SARS-CoV and MERS-CoV infections [6]. As a result of pro-inflammatory cytokine/chemokine response during SARS-CoV and MERS-CoV infections, a cytokine storm may occurs; lung epithelial and endothelial cells may undergo apoptosis [5,6]. These changes induce damaging of the alveolar–capillary barrier, alveolar edema, and disruption of gas exchange resulting in ARDS [6].

More recently, it was reported that the nucleotide sequence of SARS-CoV-2 is 79.7% identical to the SARS-CoV and 51.8% identical to the MERS-CoV [1]. As with SARS-CoV and MERS-CoV infections, in the critical patients with COVID-19 a process called 'cytokine storm' is known to play an important role in the development of ARDS and multiple organ failure [5,6]. Elevated levels in IL-1β, IFN-γ, interferon-inducible protein 10 (IP-10), and monocyte chemoattractant protein 1 (MCP-1), as well as anti-inflammatory cytokines IL-4 and IL-10, have shown in patients with COVID-19 [13].

CURRENT MEDICATIONS FOR COVID-19

Although a considerable amount of experimental researches and clinical trials have been conducted to date, currently there is no FDA-approved drug for the prevention and treatment of the COVID-19. As a result, the treatment of patients with COVID-19 is mostly based on supportive care including oxygen therapy and control of fever, prevention and/or treatment of complications, and mechanical ventilation support in severe cases [7].

A number of repurposed or investigational drugs and adjunctive therapies have been proposed for the treatment of COVID-19. Some of the most prominent repurposed drugs include chloroquine and hydroxychloroquine, lopinavir/ritonavir, umifenovir, darunavir, darunavir/umifenovir [14], darunavir/cobicistat [15], oseltamivir [16], ribavirin [17], interferon alpha-1b [18], nitazoxanide [19], ivermectin [20], and camostat mesylate [21]. Adjunctive therapies for patients with COVID-19 are consisted of corticosteroids, anti-cytokine, or immunomodulatory agents such as tocilizumab, sarilumab,

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bevacizumab, fingolimod, eculizumab, and convalescent plasma [22], high-dose vitamin C [23], dietary supplement of vitamin D [24], and ozone therapy [25]. Also, in recently published preliminary report of the Randomised Evaluation of COVID-19 therapy (RECOVERY) trial from United Kingdom (UK), easily available and cheap drug dexamethasone (6 mg/day for up to 10 days) treatment was reported to decrease 28-day mortality rates of COVID-19 patients under invasive mechanical ventilation or oxygen therapy unlike patients who do not need respiratory support [26]. Some important agents under investigation are summarized in *Figure 1*.

Chloroquine and hydroxychloroquine are FDA-approved drugs with approval dates 1949 and 1955, *respectively*, for the prevention and treatment of malaria and the treatment of systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) [22,27,28]. In addition to inhibition of viral entry and endocytosis, they attenuate cytokine production and inhibit autophagy and lysosomal activity in host cells. Chloroquine and hydroxychloroquine were reported to have *in vitro* activity against SARS-CoV-2 [22]. There are a lot of ongoing clinical trials related to the use of chloroquine and hydroxychloroquine for COVID-19 [29,30].

Chloroquine is recommended to use 500 mg twice daily for 10 days for mild, moderate, and severe cases of COVID-19 [31]. Although the optimal dose for hydroxychloroquine for COVID-19 remains unclear, according to the result of a study implementing physiologically based pharmacokinetic models, it is recommended to use a loading dose of 400 mg twice daily given orally for the first day, followed by a maintenance dose of 200 mg given twice daily for 4 days [32]. Although hydroxychloroquine has not been approved by the FDA for this indication, FDA published a fact sheet regarding as emergency use authorization of hydroxychloroquine for treatment of COVID-19 [33].

It should be kept in mind that chloroquine and hydroxychloroquine can cause QT prolongation, retinopathy, hypoglycemia, and neuropsychiatric clinical manifestations. While these drugs are compassionately used, it is should not be forgotten that QT prolongation may result in death by increasing the risk of cardiac arrhythmia. Concomitant use with other drugs (azithromycin, etc.) which prolong QT requires attention [34-37].

Lopinavir/ritonavir is an available drug combination for the treatment of human immunodeficiency virus (HIV) infection. It has been proposed against SARS-CoV-2. It is believed that lopinavir inhibits viral protease, thereby virus remains immature and loses the ability to infect cells. Ritonavir is used to prolong the duration of the effect of lopinavir by inhibiting its hepatic metabolism [38]. Lopinavir/ritonavir is proposed to use at a dose of 400 mg/100 mg twice a day for up to 14 days for COVID-19. Lopinavir/ritonavir has gastrointestinal adverse effects including nausea, diarrhea, and hepatotoxicity [22].

Darunavir, a protease inhibitor, has been used to treat HIV infection [39]. A study of molecular

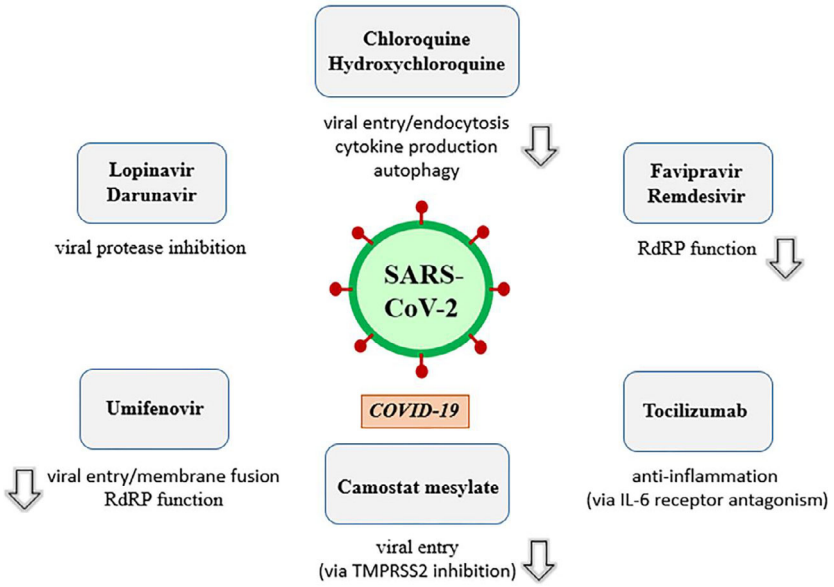

Figure 1 Important pharmacological agents under investigation against COVID-19 and their mechanism of action. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2, transmembrane serine protease 2; COVID-19, coronavirus disease 2019; RdRP, RNA-dependent RNA polymerase; IL-6, interleukin 6.

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dynamics simulations and virtual screening proposed darunavir as a repurposed drug for COVID-19 [40]. Like other antiviral agents, there is off-label use of darunavir/cobicistat for COVID-19 [41]. Cobicistat, a CYP3A inhibitor, improves oral bioavailability and reduces systemic clearance of darunavir, resulting in increased plasma concentration [42]. A phase 3 clinical trial of darunavir/cobicistat for COVID-19 is ongoing [15].

Umifenovir, also called as arbidol, is used for influenza. It has been proposed for COVID-19 based on its inhibitory effect on RNA-dependent RNA polymerase (RdRP) [43]. In addition, it targets the S protein/ACE2 interaction and inhibits membrane fusion of the viral envelope. It is under a clinical trial with oral dose of 200 mg every 8 h for COVID-19 treatment [22,44].

Camostat mesylate is used for the treatment of pancreatitis. It inhibits TMPRSS2, thereby hinders host cell entry of the virus [22].

Tocilizumab which is used for RA has off-label use for COVID-19 treatment, based on anti-inflammatory effects. It is a recombinant humanized anti-human IL-6 receptor monoclonal antibody. In a retrospective study, tocilizumab was found to be associated with improve in the symptoms, oxygenation of blood, and pulmonary opacities in patients with severe COVID-19 [45]. Tocilizumab was reported to cause abnormal liver function tests, neutropenia and anaphylaxis [46].

Remdesivir is a broad-spectrum antiviral nucleotide prodrug with potent *in vitro* antiviral activity against Ebola virus (EBOV), MERS-CoV, SARS-CoV, respiratory syncytial virus (RSV), and SARS-CoV-2 [47,48]. Remdesivir, an investigational nucleotide analogue, inhibits viral RdRP [49]. Its proposed dose is 200 mg loading dose, followed by 100 mg daily infusion for COVID-19 treatment in a clinical trial [22]. In a randomized controlled trial of Ebola virus disease (EVD) therapeutics, remdesivir was reported to may be associated with hypotension followed by cardiac arrest in a patient [50].

Favipiravir is used for influenza in Japan. It acts through its active metabolite favipiravir ribofuranosyl-5′-triphosphate, a purine nucleotide which inhibits viral RdRP. It has *in vitro* activity against SARS-CoV-2 [22]. In an open-label control study, favipiravir was given at an oral dose of 1 600 mg twice a day on first day and 600 mg twice a day on days 2–14 to patients with COVID-19. It has been reported following side effects due to favipiravir; diarrhea, liver injury, and poor diet [51].

Knowledge about proposed drugs for the treatment of COVID-19 depends on *in vitro* and animal studies as well as clinical data with low level of evidence such as case reports, case series, and clinical trials with insufficient sample size and risk of bias in the literature [22]. There is an urgent need for the development of effective prevention and treatment strategies for COVID-19. In addition, it should not be forgotten that there is also need to continuous public drug information service regarding COVID-19 treatment [52].

AN OVERVIEW OF MELATONIN

Melatonin, also called as N-acetyl-5-methoxytryptamine, is an indolamine molecule (*Figure 2*). In humans, this hormone is mainly produced by pinealocytes in the pineal gland, then released in blood. Melatonin both regulates circadian rhythm [53], and its biosynthesis in the pineal gland is correlated with the circadian rhythm which is provided by a neural system. When light stimulus reaches the retina, retinohypothalamic tract (RHT), which originates from retinal ganglion cells, sends photic signals to hypothalamic suprachiasmatic nuclei (SCN). GABAergic input originates in SCN reaches the hypothalamic paraventricular nuclei (PVN) which projects directly and indirectly to the preganglionic sympathetic neurons of the first thoracic segments of the spinal cord. Then, nerve fibers forming the conary nerves reach the pineal gland by a projection of the postganglionic sympathetic neurons which release norepinephrine, of the superior cervical ganglia (SCG). As a result, light stimulus inhibits melatonin synthesis through the suppression provided by

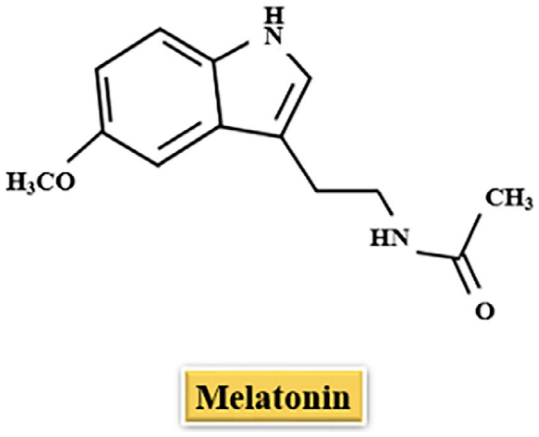

Figure 2 Chemical structure of the melatonin.

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SCN on the PVN. On the contrary, this suppression on the PVN is being removed in the dark [54,55].

Norepinephrine released by the end of the sympathetic neurons bind to α_1 and β_1 adrenergic receptors in the pinealocytes' membrane. Melatonin synthesis is induced by activation of cyclic adenosine monophosphate (cAMP)-protein kinase A (PKA)-cAMP response element binding protein (CREB) and phospholipase C (PLC)-Ca^{2+}-protein kinase C (PKC) cascades due to norepinephrine–receptor binding. Activation of these cascades leads to increase in arylalkylamine N-acetyltransferase (AANAT) which transforms serotonin to N-acetylserotonin (NAS) [54,56].

When β_1-adrenergic receptor on the pinealocyte's membrane is being stimulated, cAMP level and PKA activity increases, *respectively*, and CREB is being stimulated followed by the increase in production of N-acetyltransferase (NAT). $Alpha_1$-adrenergic receptors strengthen β_1-adrenergic activity by increasing PLC activity which leads to subsequently increase in the level of cytosolic Ca^{2+} and the activity of protein kinase C (PKC) and prostaglandins [55].

Melatonin is synthesized from tryptophan through four consecutive enzymatic reactions. First step is the conversion of tryptophan to 5-hydroxytryptophan by tryptophan hydroxylase. In the second step, 5-hydroxytryptophan is decarboxylated to serotonin. Then, serotonin is converted to N-acetylserotonin (NAS) by AANAT followed by conversion of NAS to melatonin by acetylserotonin O-methyltransferase (ASMT) [54,57].

Melatonin exerts both lipophilic and hydrophilic properties. Melatonin is not stored in the pinealocytes and can easily across the blood brain barrier depend on its amphiphilic feature. It immediately enters the cerebrospinal fluid (CSF) and blood, once synthesized in the pineal gland [53]. Melatonin binds to albumin about 70% in the blood. It has been reported to have a half-life of about 3–45 min based on its biphasic elimination when orally administered. Also, it has a half-life of about 30 min following intravenous infusion [55].

Melatonin is transformed to 6-hydroxymelatonin (6-HM) by CYP_1A_2 and conjugated to 6-sulfatoxymelatonin (6-SM) in the liver and also kidney. These metabolites eliminated through urine [54]. The main urinary metabolite of melatonin is 6-SM in humans [55].

In the central nervous system (CNS), melatonin is enzymatically, pseudoenzymatically or nonenzymatically metabolized to N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) which is converted to N-acetyl-5-methoxykynuramine (AMK) by undergoing deformylation [56]. Melatonin, AFMK, and AMK have free radical scavenging activity and protective effects against oxidative damage. AFMK is the poorest scavenger among them. Generally, protective effects against oxidative damage may be ordered as follows: AMK > melatonin > AFMK [58].

Melatonin, is known to have substantial anti-inflammatory properties and activates a number of antioxidative enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), regulates expression of several defensive enzymes and diminishes lipid peroxidation and apoptosis. It is clear that oxidative stress markedly triggers apoptosis [9]. Also, melatonin has several pleiotropic effects by distinct mechanisms. In this regard, melatonin, one of the most powerful natural antioxidants, directly interacts with reactive oxygen and nitrogen species, providing antioxidant effects independently of its cellular receptors and mobilizing the intracellular antioxidant enzymatic system. Moreover, melatonin has specific cellular membrane receptors including MT1 (MTNR1A in humans) and MT2 (MTNR1B in humans). These receptors are heterotrimeric Gi/Go and Gq/11 protein-coupled receptors which interact with adenylyl cyclase (AC), phospholipase A2 (PLA2), and PLC, resulting in reduced cAMP and cGMP production and/or increment in diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) formation. MT1 and MT2 receptors are found in nearly all peripheral tissues in addition to the CNS. Melatonin has been reported to bind to retinoid orphan receptors/retinoid Z receptors (ROR/RZR nuclear receptors) [54]. Also, melatonin has been shown to bind to a cytosolic enzyme, quinone reductase 2 (QR2), previously called as MT3 receptor [59].

EXPERIMENTAL USE OF THE MELATONIN AGAINST VIRAL INFECTIONS

Melatonin has been reported to be effective against several viral infections [60]. Melatonin exerts indirect antiviral properties by reducing inflammation and oxidative stress, and also by modulating immune responses [5].

Ebola virus disease is a rapidly progressive and highly lethal disease caused by EBOV. The most important cause of death due to EVD is hemorrhagic shock syndrome (HSS) [61]. There is currently no FDA-approved antiviral agent for the treatment of patients with EVD. Although there are investigational antiviral drugs such

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as regeneron (REGN-EB3) and mAb114 for EVD, treatment mainly remains supportive [62]. EBOV causes severe vascular leakage also called as Ebola hemorrhagic shock syndrome (EHSS) by activating the Rho/Rho-associated protein kinase (Rho/ROCK) pathway which leads to actin bundle formation and a tensile force relaxing the junctions between the vascular endothelial cells (VECs). Recently, melatonin was found to effective against Ebola hemorrhagic shock syndrome in an engineered microvessel-on-a-chip model. In this study, melatonin was observed to reduce vascular permeability by inhibiting Rho/ROCK signaling in VECs [61].

Respiratory syncytial virus may lead to severe lung disease because of excessive inflammatory immune response in childhood. In this regard, oral pre-administration of melatonin at dose of 5 mg/kg twice daily for 3 days was shown to result in a significant decrement of oxidative lung damage and inflammation in mice infected with RSV. It was reported that melatonin, markedly inhibited the increment of malondialdehyde (MDA), nitric oxide (NO), and hydroxyl radical (·OH) levels and restored the reduced glutathione (GSH) and SOD levels in the lung due to its antioxidant and free radical scavenger effects. Also, melatonin significantly inhibited inflammation by reducing elevated level of serum TNF-α [63]. The protective effect of melatonin against RSV infection has also been shown *in vitro* study. According to the result of this study, melatonin pre-treatment was observed to inhibit the elevation of toll-like receptor 3 (TLR3)-mediated inflammatory gene expression including nuclear nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB), TNF-α and inducible nitric oxide synthase (iNOS) without affecting TLR3 protein, in RSV-infected macrophages. It was suggested that melatonin suppresses the elevation of TNF-α and iNOS expression by inhibiting NF-κB nuclear translocation [64].

Venezuelan equine encephalitis (VEE) is a viral infection caused by VEE virus (VEEV) which especially affects human and equines. The inflammation induced by VEEV is associated with a high mortality rate in mice. According to data obtained from an experimental study, while melatonin application for pre-treatment (three days before the infection and continuing until the end of the experiment) and treatment (during the infection) increased the survival rate, melatonin application for post-treatment (24 h after the infection) was found to be ineffective on survival in mice infected with VEEV, suggesting melatonin has a preventive effect on VEE infection. Melatonin administrated at a dose of 500 mcg/kg by subcutaneous (sc) injection in all *in vivo* experiments of mentioned study. Melatonin reduced the apoptosis in the brain of infected mice and in the VEEV-infected neuroblastoma cells. On the contrary, melatonin was reported to cause apoptosis of uninfected neuroblastoma cells [65]. Melatonin was also shown to protect mice infected with the VEEV by decreasing mortality rate, postponing the onset of the disease, and reducing viral load in the brain as well as in the blood. Also, it has been reported that melatonin diminishes the cell destruction in the chicken embryo fibroblasts infected with the VEEV [66].

Melatonin also was shown to have protective effect against acute liver failure caused by rabbit hemorrhagic disease virus (RHDV) in an experimental study. It was reported that melatonin exerts anti-inflammatory effect including decrement in TNF-α and IL-6, and decreases viral replication by inhibiting hepatic sphingosine kinase 1 (SphK1)/sphingosine-1-phosphate (S1P) signaling pathway in rabbits infected with RHDV [67].

Melatonin also was reported to protect minks from Aleutian disease (AD) is a viral disease which leads to lesions in the kidney, liver, lungs, and arteries because of hypergammaglobulinemia and immune complexes caused by AD parvovirus. Melatonin was observed to markedly reduced mortality rate in minks infected with AD virus. It was suggested that protective effect of melatonin may be due to its free radical scavenger, immunomodulator, and antioxidant enzyme-inducing properties [68].

Influenza virus causes respiratory tract infections likely by apoptosis and production of reactive oxygen species (ROS) in the airway epithelial cells. Melatonin exerts protective and therapeutic effects against influenza virus. In a recent study, efficacy of melatonin was investigated on a murine model of influenza A infection. Melatonin was administered as prophylactically (20 mg/kg or 200 mg/kg sc) or therapeutically (200 mg/kg sc). Prophylaxis or treatment with 200 mg/kg melatonin increased survival rate. Treatment with 200 mg/kg melatonin was reported to result in reduced levels of TNF-α, IL-6, and IFN-γ, and elevated levels of IL-10 and TGF-β in bronchoalveolar lavage fluid (BALF) reflecting production in lung. In addition, melatonin treatment was reported to decrease pulmonary leukocyte infiltrates and the phosphorylation of NF-κB p65 in the lung homogenate of mice, suggesting reduced levels of pro-inflammatory cytokines associated with reduced activation of NF-κB. According to this study, supplementation of melatonin

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to ribavirin treatment resulted in higher survival rate than ribavirin alone [69]. On the contrary, long-term dietary supplementation of melatonin was reported not to have significant effect on lung IL-1β, IL-6, and TNF-α levels, plasma IL-6 level, H_2O_2 production by lung cells, liver 4-hydroxynonenal (4-HNE), and MDA levels, pulmonary viral titers, preventing the weight loss and decreased food intake in mice infected with influenza [70]. The lack of studies makes it difficult to comment on whether melatonin is effective in the prophylaxis or treatment of influenza. More studies are needed to elucidate the beneficial effects of melatonin on influenza infection.

According to aforementioned data obtained from experimental studies in the literature, melatonin may be a potential therapeutic agent for the prevention of disease development and reducing disease severity as well as mortality rates in numerous viral infections.

MELATONIN AND COVID-19

Oxidized products are released during viral infections and replication [5]. Oxidative damage and inflammation have an important role in the pathogenesis of viral infections. Damage of many organs may occur as a result of these pathological processes [1]. Melatonin, a powerful endogen antioxidant, free radical scavenger, and anti-inflammatory molecule, has been reported to exert beneficial effects on viral diseases [60]. In addition, the effectiveness of melatonin has been demonstrated in many different conditions associated with inflammation ischemia–reperfusion (I/R) injury and oxidative stress. Our results showed that physiological melatonin concentration has an important role in the protection from I/R injury in the body [71]. In this context, we previously observed that melatonin is a protective agent against liver damage, induced by pinealectomy, renal I/R or myocardial I/R [72–74], I/R injury of brain [75], heart [76] and flap injury [77], testicular injury [78], radiation damage [79], cerebral vasospasm after subarachnoidal hemorrhage [80] and colitis [9] in rats. In this context, there are several possible conditions increasing free radicals in COVID-19 patients as follows: excessive inflammation, cytokine storm, hypoxemia, and respiratory support by mechanical ventilation. It is well known that mechanical ventilation can cause ventilator-induced lung injury (VILI) which includes alveolar damage, lung edema, accumulation of immune cells, pro-inflammatory cytokine discharge, and the exaggerated ROS generation. Unfortunately, VILI is a condition which can result in sequelae and even death. Recently, ramelteon, a melatonin receptor agonist, has been reported to protect lung from VILI by increasing IL-10 production in a rat model [81]. Decreased level of intracellular heme oxygenase 1 (HO-1), an antiviral, anti-inflammatory, antioxidant, and cytoprotective stress protein, may exert an important role in the pathogenesis of COVID-19 [82]. Melatonin is known to increase a group of antioxidant proteins including HO-1 by activating NF-E2-related factor 2 (Nrf2) [83,84]. In this regard, melatonin was proposed to use for COVID-19 treatment because of its HO-1 enhancing property [82]. According to our experiences and available literature data, it seems to us that melatonin may also be beneficial in COVID-19 treatment [5], and anti-inflammatory properties COVID-19 blockade of heme synthesis could limit the HO-1 stress tolerance function, contributing to host fragility.

As a good news, recently, melatonin was proposed to be a potential candidate drug as an adjuvant treatment for patients with COVID-19 [5]. Also, melatonin was found to be candidate drug for COVID-19 in a network-based drug repurposing study. Melatonin was predicted to indirectly interact with the human CoVs-associated cellular proteins, including ACE2, B-cell lymphoma 2 (Bcl-2)-like protein 1 (BCL2L1), JUN, and inhibitor of NF-κB kinase subunit beta (IKBKB). Also, according to this study, combination of mercaptopurine and melatonin may be a potential treatment for COVID-19 by synergistically targeting papain-like protease, ACE2, c-Jun signaling, and anti-inflammatory cascades [1].

The possible anti-inflammatory effects of melatonin that may be beneficial in the ALI/ARDS induced by COVID-19 involves upregulation of sirtuin-1 (SIRT1), suppression of NF-κB activation, and stimulation of NF-E2-related factor 2 (Nrf2), thus a decrement in the pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8) and increment in the level of anti-inflammatory cytokine IL-10 [5]. In addition, melatonin exerts cardioprotective effects by modulating apoptosis and autophagy via elevating expression of SIRT1 in septic mice [85]. Preclinical and clinical studies have reported that melatonin reduces pro-inflammatory cytokines in many diverse conditions. In this regard, melatonin has been reported to dose-dependently reduce TNF-α and IL-1β production but not IL-6 in human RA synovial fibroblasts [86]. Melatonin administration (orally 25 mg daily for 6 months) has been reported to reduce

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serum levels of TNF-α, IL-1β, IL-6, lipoperoxides, and NO catabolites in patients with relapsing–remitting multiple sclerosis (RRMS) [87]. In a double-blind, placebo-controlled clinical trial, melatonin (orally 6 mg daily for 8 weeks) administration significantly decreased serum level of IL-6 and high-sensitivity C-reactive protein (hs-CRP) but not TNF-α in type 2 diabetes mellitus (DM) patients with chronic periodontitis [88]. Melatonin has been reported to reduce TNF and IL-6 levels, and increase IL-10 level in human placental trophoblasts which have been undergone hypoxia/reoxygenation [89]. Melatonin administration (10 mg at 09:00 h and 60 mg at 21:00 h for 3 months) has been reported to reduce plasma levels of IL-1β, IL-2, IL-6, and TNF-α in patients with Charcot–Marie–Tooth Neuropathy [90]. In a double-blind, placebo-controlled clinical trial, melatonin administration (6 mg daily for 40 days) has been reported to reduce plasma levels of TNF-α and IL-6 in obese women [91]. Melatonin administration (25 mg/kg ip 30 min before each caerulein injection) has been reported to result in decrease in IL-1β and TNF-α, and increase in IL-4 serum levels in rats with caerulein-induced acute pancreatitis [92]. Melatonin administration (10 mg/kg ip) has been reported to reduce TNF-α serum level in rats with Escherichia coli-induced pyelonephritis [93]. These data of *in vivo*, *in vitro,* and clinical studies indicate that melatonin may be potential supportive agent in counteracting with cytokine storm in patients with COVID-19.

Melatonin improves proliferation and maturation of natural killer (NK) cells, T and B lymphocytes, granulocytes, and monocytes, thus supports immune response. Melatonin augments antigen presentation in macrophages; thus, complement receptor 3, MHC class I and class II, and CD4 antigens are upregulated. Given the level of neutrophils, lymphocytes and $CD8^+$ T cells may decrease in peripheral blood in COVID-19 patients, melatonin may also be useful as an immunoregulator [5]. Also, melatonin has been reported to blunt the NF-kB induction and decrease the NLRP3 expression in heart of mice with polymicrobial sepsis induced by the cecal ligation and puncture (CLP) model. It is well established that the nucleotide oligomerization domain (NOD)-like receptor 3 (NLRP3) inflammasome plays an important role in the innate immune response during inflammatory states [94]. In addition, melatonin has been reported to reduce the macrophage and neutrophil infiltration in the lung by inhibiting NLRP3 inflammasome in experimental models. This effect may be another reason for using melatonin in the treatment of COVID-19 [5].

Currently, in Spain, there is an ongoing multicenter randomized placebo-controlled phase 2/3 clinical trial 'MeCOVID' investigating whether melatonin has an efficacy in the prophylaxis of COVID-19 among healthcare workers. Status of this trial is not yet recruiting. Four hundred fifty participants between the ages of 18–65 are estimated to be included in the study. It is known that that the peak blood level of melatonin is higher in younger children and SARS-CoV-2 appears to less affect them when compared to other groups of age. In this regard, the researchers supposed that approximately reaching the melatonin levels in children may protect from infection or hinder progression to severe disease even if the infection occurs. For this purpose, melatonin with prolonged release will be administered orally at a dose of 2 mg daily before bedtime for 12 weeks. Confirmed symptomatic infection rate will be considered as primary outcome measure of the study [95].

Inhibition of melatonin by most viruses suggests that modulation of melatonin may be useful in the management of viral infections [96]. Although melatonin has not direct effects on viral replication or transcription, based on its antioxidant and anti-inflammatory properties, it may be a potential drug to reduce the severity of clinical symptoms. Melatonin also may decrease mortality rate among patients with viral disease and save them time to recover [1]. Given the antioxidant and anti-inflammatory effects, melatonin seems to be a potential agent for attenuation of COVID-19 infection. As an adjuvant therapeutic agent, melatonin may be useful in COVID-19 and related complications including ALI and ARDS likely by immune regulation, anti-inflammation, and antioxidation [5]. There is urgent necessity to a lot of well-designed preclinical and clinical studies investigating efficacy of melatonin for COVID-19 treatment.

MELATONIN-RAS RELATIONSHIP: BENEFITS FOR COVID-19

ACE2, a homologue of ACE, is found as two types: membrane-bound and soluble. Membrane-bound ACE2 is a type I transmembrane metallopeptidase which comprises an extracellular catalytic domain, receptor for SARS-CoV-2, and a transmembrane anchor. Soluble ACE2 circulates in the blood and has no anchor [97]. ACE2 operates as a monocarboxypeptidase with its extracellular catalytic domain which degrades angiotensin II (Ang II) and angiotensin I (Ang I) into

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angiotensin 1–7 (Ang 1-7) and angiotensin 1-9 (Ang 1-9), respectively [98]. In addition, ACE2 possibly exerts enzymatic effect on other substrates including apelin, des-arginine bradykinin, and neurotensin. Ang 1-7–mitochondrial assembly (MAS) receptor binding leads to vasodilation, anti-inflammatory, and anti-fibrotic effects. Thus, Ang 1-7 establishes the balance by eliminating the harmful effects caused by Ang II [97].

ACE2 is located primarily in the lung (airways and type II alveolar cells), heart, kidney, and intestine. It is also found in oral and nasal mucosa, skin, lymph nodes, thymus, bone marrow, spleen, liver, testis, and brain [97].

Endocytosis of viral particles–ACE2 complex leads to decrease in membrane-bound ACE2 expression. In addition, upregulation in protease activity of a disintegrin and metalloproteinase 17 (ADAM17) which separating 2 domains of ACE2 from each other results in sheds extracellular catalytic domain of ACE2 into the circulation. Thus, while activity of the protective ACE2/Ang 1-7/MAS receptor axis is decreasing, increased activity of harmful ACE/Ang II/ Angiotensin II receptor type 1 (AT1 receptor) axis is occurred. Increased level of Ang II leads to further upregulation of ADAM17 activity through the AT1 receptors and downstream extracellular signal-regulated kinase (ERK)/ p38 mitogen-activated protein kinase (MAPK) signaling pathways. ADAM17 also associated with the liberation of membrane-bound precursors of TNF-α, IFN-γ, and IL-4 into the circulation. It is well established that IL-4 and IFN-γ reduce ACE2 expression [99. Therefore, reduction in tissue ACE2 levels may result in increased lung damage and tissue fibrosis seen in COVID-19 cases [97]. COVID-19 not only affects the lungs but also causes acute cardiac and renal damage, myocarditis, arrhythmias, and gut and liver pathologies. These events are considered to be related to the loss of tissue ACE2 as a result of COVID-19 [99]. In this context, SARS-CoV infection is also known to cause cardiac dysfunction, arrhythmias, and even cardiac death. SARS-CoV infection has been reported to cause myocardial dysfunction by reducing myocardial ACE2 expression in mice [96]. Remarkably high Ang II plasma levels which correlated with viral load and pulmonary damage have been reported in patients with COVID-19 [100]. This increase in Ang II levels in patients with COVID-19 seems to be result of a decrease in tissue ACE2 levels. Also, reduced plasma Ang II/Ang 1-7 ratios have been reported in recombinant human ACE2 (rhACE2)-treated patients with pulmonary arterial hypertension and ALI. Furthermore, ACE inhibitors and ARBs have protective effects against dramatic results of cardiovascular disease partially by elevating ACE2 levels [99]. Based on this relationship, it has been recommended that patients under ACEI and ARB treatment should not discontinue their medication during COVID-19 pandemic if there is no clinical indication [101]. Soluble form of ACE2 has been reported to block SARS-CoV replication in the monkey kidney cell line. In addition, SARS-CoV-2 has been reported to be neutralized by ACE2 fused to the Fc portion of immunoglobulin. In this regard, soluble rhACE2 has been proposed to be useful in COVID-19 treatment by preventing SARS-CoV-2–membrane-bound ACE2 interaction [102]. In this regard, an open-label, randomized, and controlled clinical trial was started in China. In this study, it was planned to intravenously administrate 0.4 mg/kg rhACE2 twice a day for 7 days. However, this study was withdrawn due to lack of Center for Drug Evaluation (CDE) approval before participants were enrolled [103].

There is an ongoing open-label, randomized, and controlled phase 1 clinical trial in Egypt. In this study, it is aimed to investigate the potential beneficial effects of recombinant bacterial ACE2 receptors-like enzyme of B38-CAP (rbACE2) on an estimated 24 adult patients with COVID-19. The study has not started to be recruited yet. 0.4 mg/kg rbACE2 will be intravenously administrated to the intervention group twice a day for 7 days [104].

According to the aforementioned preclinical and clinical data, reduced tissue expression of ACE2 and thus shift of RAS balance toward ACE/Ang II/AT1 pathway seem to be an important factor in the pathogenesis of multiple organ damage including lung, heart, kidney, and liver in patients with COVID-19. These data suggest that restoring tissue ACE2 levels and also preventing shift of RAS balance toward ACE/Ang II/AT1 pathway may be an effective approach in the treatment of COVID-19. RAS modulating effects of melatonin has been reported in several experimental studies (*Table I*).

Maternal melatonin application (0.01% melatonin in drinking water during pregnancy and lactation) has been reported to increase ACE2 and AT2R expression as well as MAS receptor protein levels in the kidney of male offspring rats with prenatal dexamethasone (DEX)-induced programmed hypertension. In this study, investigators have suggested that elevated renal MAS protein levels play an important role in the prevention of DEX-induced programmed hypertension by melatonin [105].

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Table I Effects of melatonin on RAS components in *in vivo* experiments.

Investigators	Disease model	Animals	Dosing	Effects
Tain, Y. L. *et al*. [105]	Programmed hypertension (PH) induced by prenatal dexamethasone (DEX) administration	Female 12-16 weeks old Sprague–Dawley (SD) rats	0.01% melatonin in drinking water during pregnancy and lactation	Increase in ACE2 and AT2R expression as well as MAS receptor protein levels in the kidney of male offspring
Wu, T. H. *et al*. [106]	PH induced by neonatal DEX administration	Male neonate offspring of female 12-16 weeks old SD rats	0.01% melatonin in drinking water during the lactation period	Increase in ACE2 expression in the kidney and heart of male offspring
Tain, Y. L. *et al*. [107]	PH induced by maternal caloric restriction	Female 12-16 weeks old SD rats	0.01% melatonin in drinking water during pregnancy	Increase in ACE2 expression and protein levels in the kidney of the male offspring
Tain, Y. L. *et al*. [108]	PH induced by maternal exposure to continuous light	Female 12-16 weeks old SD rats	50 mg/day ip agomelatine during pregnancy and lactation 0.01% melatonin in drinking water during pregnancy and lactation	*Agomelalatine* Decrease in expression of ACE and ACE2 Increase in expression of AT2 receptor and MAS receptor in kidney. *Melatonin* Decrease in renal ACE expression.
Tain, Y. L. *et al*. [109]	PH induced by prenatal DEX and postnatal high-fat diet	Female 12-16 weeks old SD rats	0.01% melatonin in drinking water during pregnancy and lactation	Increase in renal expression of AT2 receptor and MAS receptor in male offspring

Melatonin administration to pups (0.01% melatonin in drinking water during the lactation period) has been reported to increase ACE2 expression in the kidney and heart of male offspring rats with neonatal DEX-induced programmed hypertension [106]. Maternal melatonin treatment (0.01% melatonin in drinking water during pregnancy) increased ACE2 expression and protein levels in the kidney of the adult offspring exposed to maternal caloric restriction [107].

In an experimental study investigating effects of maternal agomelatine (melatonin receptor agonist) and melatonin treatment during pregnancy and lactation on programmed hypertension in male offspring rats of mother exposed to continuous light, several changes have been reported on RAS. In this study, it has been reported that maternal agomelatine (50 mg/day ip) administration decreased expression of ACE and ACE2 but increased expression of ang II type 2 receptor (AT2 receptor) (mediating beneficial effects of Ang II) and MAS receptor in kidney. Additionally, maternal melatonin application (0.01% in drinking water) reduced renal ACE expression (108).

Maternal melatonin therapy (0.01% melatonin in drinking water during pregnancy and lactation) has been reported to increase renal expression of AT2 receptor and MAS receptor in prenatal DEX and postnatal high-fat diet-induced programmed hypertension in male offspring rats [109].

CONCLUSION

Considering the limited number of studies mentioned above, melatonin may be a potential agent to prevent multiple organ injuries and subsequent disease progression as well as sequelae in patients with COVID-19 due to its modulating effects on RAS, and antioxidant, anti-inflammatory, free radical scavenger, and antiviral and immunomodulatory effects. Ultimately, the results of the ongoing trial of melatonin in the prophylaxis of COVID-19 on healthcare workers and additional clinical and preclinical studies will offer more strong evidences to science world.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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