Review

Melatonin: A potential therapeutic agent against COVID-19

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Running title: Melatonin and COVID-19

Received: September 11, 2020; Accepted: October 18, 2020

ABSTRACT

Coronaviruses (CoVs) are RNA viruses that cause infections of the respiratory, gastrointestinal, and central nervous systems, among others. The pathological symptoms of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) include excessive inflammation, elevated oxidative stress, and an exaggerated immune response, ultimately leading to a cytokine storm and subsequent progression to acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) and often, death. Melatonin is a multifunctional and highly significant biomolecule that has anti-inflammatory, anti-oxidative, anti-apoptotic, and neuroprotective actions with no serious undesired side effects, even when administered in high doses. In this review, we present a brief account of the origin of coronaviruses, their characteristic features, infections, transmission, and the causes of coronavirus disease 2019 (COVID-19). We discuss their structure, genome organization, and mechanisms of cellular entry, as well as the pathogenicity of severe acute respiratory syndrome (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and SARS-CoV-2. Furthermore, we provide an account of the typical characteristic features of melatonin, such as its antioxidant, anti-inflammatory, immunomodulatory, and ameliorative effects on various virus-induced infections. Additionally, we identify the rationale for using melatonin as both a prospective adjuvant with vaccine therapy, and as an antiviral immune stimulator. Finally, we provide a perspective on the use of melatonin as a treatment against COVID-19.

Key words: Coronavirus (CoVs), melatonin, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome coronavirus (MERS); severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), coronavirus disease 2019 (COVID-19), antiviral agent.

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1. INTRODUCTION

Coronaviruses (CoVs) are a large family of potentially pathogenic RNA viruses responsible for the currently emerging respiratory disease, CoV disease 2019 (COVID-19). These viruses cause a

variety of diseases in both mammals and birds (1), and are able to cross species barriers, causing serious respiratory pathologies in humans, such as middle east respiratory syndrome CoV (MERS), severe acute respiratory syndrome (SARS), and COVID-19. The structure of their spike proteins gives them the appearance of a crown; therefore, these viruses are referred to as CoVs. Disease symptoms differ between species, for example, chicken CoVs not only attack the respiratory tract, but also the urogenital system, potentially spreading to other organs as well (2). In porcine and bovine species, however, CoVs cause serious diarrhea.

Phylogenetic analysis has revealed that SARS-CoV-2 is related to SARS-like bat viruses; thus, bats are likely its primary reservoir. COVID-19 has become a pandemic, with 35.7 million people being infected (3, 4). Its clinical symptoms include acute respiratory disorder induced by either highly homogenous CoVs or other pathogens (5). Evidence suggests that excessive proinflammatory and oxidative responses contribute to the pathology of COVID-19, leading to a cytokine storm and subsequent progression to acute lung injury (ALI)/acute respiratory distress syndrome (ARDS), and often, death (5).

COVID-19 is caused by a novel coronavirus designated as SARS-CoV-2 (4, 6). SARS-CoV-2 has 80% and 50% homology with SARS-CoV and MERS-CoV, respectively (3, 4). CoVs are thought to be a primarily cause of enzootic infections in birds and mammals. Repeated infections with CoVs have caused a series of diseases to human including SARS, MERS, and now, COVID-19. Data suggest that CoVs have the potential to cross species barriers and be transmitted among humans (7). Currently, there is no reliable treatment for COVID-19, which contributes both to its high degree of spread and rate of mortality. The prolonged wait for an effective vaccine against COVID-19 is unavoidable, unfortunately leading to both a devastating loss of human life and decimation of the global economy.

Melatonin is a pineal hormone primarily synthesized and secreted during the night from its essential precursor, tryptophan (8, 9). It is also produced in other tissues, including bone marrow cells, lymphocytes, thymus, heart, muscle, spleen, liver, stomach, intestine, and epithelial cells (10). Mitochondria produce melatonin and also regulate GPCR signaling to block cytochrome c release (11). Once formed in the pineal gland, melatonin is quickly released into the cerebrospinal fluid and blood (12). Initially identified as a skin-lightening agent in amphibians, later studies demonstrated that melatonin influences circadian rhythms and seasonal reproduction, as well as protects the placenta, fetus, and mother from oxidative damage caused by a variety of pregnancy-associated toxic oxidizing events (13-15). Melatonin and its metabolites also play critical roles in immunomodulation, also possessing antioxidative capabilities, owing to their direct and indirect ability to scavenge reactive oxygen species (ROS) (16, 17). Subsequent research has documented melatonin as a highly resourceful, multifaceted pleiotropic agent that orchestrates countless physiological functions (9, 18-20). It regulates some functions through membrane-bound MT1 and MT2 and widely distributed G protein-coupled receptors (21-23), while other actions seem to be receptor independent, for example, direct free radical scavenging. A third cytosolic receptor, MT3, is involved in protecting against oxidative stress, through the prevention of quinone electron transfer reactions (24). Resveratrol, imatinib and nilotinib exhibit high affinity towards NQO2 (25, 26). Melatonin's role in the nucleus does not require physical interaction between melatonin and RORα (27).

COVID-19 causes various levels of impaired consciousness, ranging from somnolence to confusion, delirium, stupor, and coma, in almost 15% of hospitalized patients. Major pathogenic mechanisms of delirium occur due to a variety of factors, including neurotransmitter imbalance, pro-inflammatory cytokines, hypoxia, and sleep deprivation. Delirium occurred in up to 50% of

hospitalized elderly patients and 80% of critically ill patients receiving mechanical ventilation (28, 29). Administration of melatonin or melatonin receptor agonists (MRAs) reduced delirium and improved sleep quality in intensive care unit (ICU) patients (30, 31). Melatonin can reduce the molecules responsible for worsening both delirium in the elderly, and central respiratory depression, such as benzodiazepines or antipsychotics. With unique multifarious effects, such as anti-inflammatory, antioxidative, and immune-enhancing activities, melatonin may assist in alleviating infection-induced acute respiratory distress (5). Studies using animal models have demonstrated that melatonin can ameliorate ALI by acting as a direct antioxidant effect or melatonin receptor activation. The protective effect of ramelteon, a melatonin receptor agonist (MRA), against ventilator–induced lung injury has recently been shown to depend on the upregulation of interleukin (IL)-10 in rats (32). PAK1 (RAC/CDC42-activated kinase 1) is the main "pathogenic" kinase whose abnormal activation causes a wide variety of diseases/disorders, including cancers, inflammation, malaria, and pandemic viral infections, including influenza, HIV, and COVID-19. Melatonin can be used as a PAK1 blocker, similar to propolis, cyclesonide, hydroxychloroquine (HQ), ivermectin, and ketorolac (33). Its supplemental dose may overcome SARS-COV-2-induced infections by reversing aerobic glycolysis through the repression of both HIF-1α and mTOR, thereby disinhibiting pyruvate dehydrogenase complex (PDC) activity and allowing acetyl-coenzyme A synthesis (34). Combining mitochondrion-produced and parenteral melatonin can reduce the cytokine storm, as well as relieve COVID-19 infection-induced damage (35). The important aspect that needs to addressed is cross-contamination during cryopreservation which is due to presence of SARS-CoV-2 on tissues, gametes and embryos (36). MLT can be used as a fertility-friendly anti-coronavirus agent (37)

Based on available published literature, the current review focuses on the origin, characteristics, infection rate, and transmission of human CoVs. In addition to details of the pathogenicity of SARS-CoV, MERS-CoV, and SARS-CoV-2, we provide an account of the structure, genome organization, and mechanisms of cellular entry of CoVs. Furthermore, we outline the synthesis, metabolism, and biological functions of melatonin, such as its anti-inflammatory and antioxidative activities, as well as its ability to inhibit inflammatory and immune responses. We also discuss the associated infectiousness, biological features, and beneficial effects of melatonin against various types of viral infections, including influenza, Ebola virus disease, SARS-CoV, MERS-CoV, and SARS-CoV-2. Finally, we evaluate the justification for using melatonin as a prospective adjuvant to vaccine therapy, as well as the antiviral immune features of this endogenously produced indoleamine.

2. STRUCTURE AND GENOME ORGANIZATION OF COVS

CoVs, ranging between 65 and 125 nm in size, belong to the *Coronaviridae* family in the order *Nidovirales*. Their genetic material is composed of single stranded RNA, with an average size between 26 and 32 kb in length. The World Health Organization (WHO) has classified SARS-CoV-2 as a β-CoV of subgroup 2B (38), with its genetic sequence sharing more than 80% identity with that of SARS-CoV and 50% identity with that of MERS-CoV (39, 40), both of which originated in bats (41). Phylogenetic analysis has revealed that SARS-CoV-2 belongs to the genus β-CoV, which includes SARS-CoV, infecting humans, bats, and other wild animals (6).

Characteristic features of CoVs include specific genes in their open reading frame 1 (ORF1) downstream regions that encode proteins for viral replication, and nucleocapsid and spike formation (42). Glycoprotein spikes on their outer surface are responsible for viral attachment and

their entry into host cells. The main reason SARS-CoV-2 contributes to multiple infections in the host is weak receptor-binding domain (RBD) attachment (43). While other CoVs use aminopeptidases or carbohydrates as key receptors for their entry into human cells, SARS-CoV and MERS-CoV, whose genomes are 29,727 and 30,119 nucleotides in length, respectively, recognize exopeptidases (44, 45). The SARS-CoV *rep* gene accounts for approximately two-thirds of the genome, encoding for at least two polyproteins that undergo cotranslational proteolysis (46). The MERS-CoV genome does not encode for a hemagglutinin-esterase (HE) protein (47). Genomic analysis of MERS-CoV revealed that its outbreak was likely due to genetic recombination (48). MERS-CoV and SARS-CoV possess five and eight accessory proteins, respectively, which facilitate entry into humans and cause harmful effects on the immune system. Differences in proteins between the two viruses accounts for the greater sensitivity of MERS-CoV and SARS-CoV to the induction of interferon (IFN) production and type 1 IFN signaling (49)

A typical CoV contains at least six ORFs in its genome, in which four structural genes encode four structural proteins, including spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins (Figure 1A). *Orf1ab* is the largest gene in SARS-CoV-2, encoding for the pp1ab protein and 15 nonstructural proteins (nsps). The first ORF (ORF1a/b) encodes 16 nsps (40, 50, 51) and occupies two-thirds of the entire genome length. ORF1a and ORF1b produce two polypeptides, pp1a and pp1ab**.** Through either virally encoded chymotrypsin-like protease (3CLpro) or main protease (Mpro), and one or two papain-like proteases, these polypeptides are processed into 16 nsps. All structural and accessory proteins are translated from CoV sgRNA. ORFs 10 and 11 encode four main structural proteins and occupy one-third of the genome near the 30-terminus (42, 52). These four main structural proteins encode special structural and accessory proteins, such as HE, 3a/b, and 4a/b proteins**.** These mature proteins are responsible for several important functions, including genome maintenance and virus replication (42). Interestingly, to maintain van der Waals forces, SARS-CoV-2 spike proteins contain a 3-D structure in the RBD region (53). Glutamine residue 394 of the RBD region is recognized by lysine residue 31 of the human angiotensin converting enzyme 2 (ACE2) receptor, with these two regions playing a critical role in host-virus interactions (54). Evolutionary data indicates that SARS-CoV-2 lies close to the SARS-CoV group (38, 55). CoVs have four different subgroups including alpha, beta, gamma, and delta **(**Figure 1B).

Recent studies have shown significant variations between SARS-CoV and SARS-CoV-2, such as absence of protein 8a and fluctuations in the number of amino acids in proteins 8b and 3c in SARS-CoV-2 (50, 56). Furthermore, the glycoprotein spike of SARS-CoV-2, which is a combination of bat SARS-CoV and an unknown β-CoV (55), was found to be modified via homologous recombination. Notably, a single N501T mutation in the SARS-CoV-2 spike protein could potentially have influenced the affinity for its binding to ACE2 on host cells (54). Both 5′ and 3′ UTRs play critical roles in inter- and intramolecular interactions, RNA-RNA interactions, and binding of viral and cellular proteins (57). When comparing the 5′ end of SARS-CoV-2, SARS-CoV, and MERS-CoV, Pb1ab is the first ORF of the entire length of genome to encode non-structural proteins with a size of 29844 bp (7096 aa), 29751 bp (7073 aa) and 30119 bp (7078 aa), respectively (Figure 1C). Considering the 3′ end spike proteins, these three β-CoVs differ notably with regard to the length and number of amino acids, i.e., the presence of 1273 aa, 21493 aa, and 1270 aa in SARS-CoV-2, SARS-CoV, and MERS-CoV, respectively (56, 57). A recent study showed that SARS-CoV-2 *ORF3b* is a potent interferon antagonist, suppressing the induction of type I interferon more efficiently than its SARS-CoV ortholog; the paper also described the details of 9 ORFs (58).

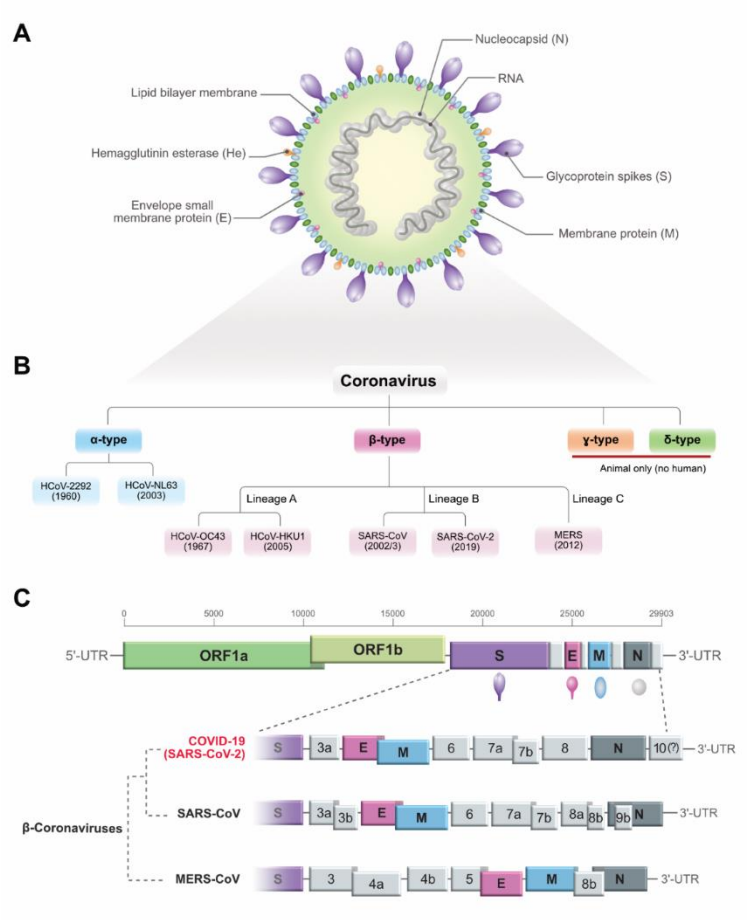


Fig. 1. Structure and genome organization of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

(A) SARS-CoV-2 is a spherical or pleomorphic enveloped particle containing single-stranded (positive-sense) RNA associated with a nucleoprotein within a capsid. (B) Coronaviruses (CoVs) have four different subgroups, including alpha, beta, gamma, and delta. Among them, seven α- and β-derived CoVs cause disease in humans, while γ and δ infect animals only. (C) Genomes of human β-CoVs (SARS-CoV, MERS-CoV, and SARS-CoV-2,) include an untranslated region (UTR), open reading frame (ORF), and structural proteins, including spike, envelope, membrane, and nucleocapsid. CoVs contain glycoprotein spikes on their outer surface, which are responsible for attachment to, and entry into, host cells.

3. PATHOGENICITY OF COVS

Phylogenetic analysis has revealed that the SARS-CoV-2 genome sequence is 96.2% identical to the bat CoV RaTG13; in comparison, it shares 79.5% identity to SARS-CoV (4, 40, 59). Sequence analysis suggests that bats may be natural hosts for the origin of this virus, and that COVID-19 may be transmitted from bats, via unknown intermediate hosts, to infect humans (59). A structural model study predicted that the binding affinity of SARS-CoV-2 for ACE2 is more than 10-fold higher than that of SARS-CoV (60). Cryo-EM structure of the SARS-CoV-2 spike demonstrated that it has faster transmission capabilities and is responsible for higher numbers of

confirmed COVID-19 cases in humans than SARS-CoV. Collectively, these factors suggest that SARS-CoV-2 has higher affinity for binding to ACE2, indicating that soluble ACE2 may be a potential candidate for COVID-19 treatment (60, 61). Patients with COVID-19 exhibit various clinical manifestations, similar to the symptoms of SARS-CoV and MERS-CoV infections. CoVs, such as SARS-CoV-2, are pathogenic when their spike proteins bind to ACE2, allowing them to enter and infect host cells.

After binding to the host-receptor cell, SARS-CoV-2 fuses with the cellular membrane and releases genetic material into the nucleus or cytoplasm. Next, viral RNA is transcribed, thereafter directing protein synthesis. The virus replicates and assembles into new virions, which are then released via exocytosis into the vicinity of neighboring cells. CoV genome replication, occurring in the cytoplasmic membrane, involves coordinated processes of both continuous and discontinuous RNA synthesis (Figure 2).

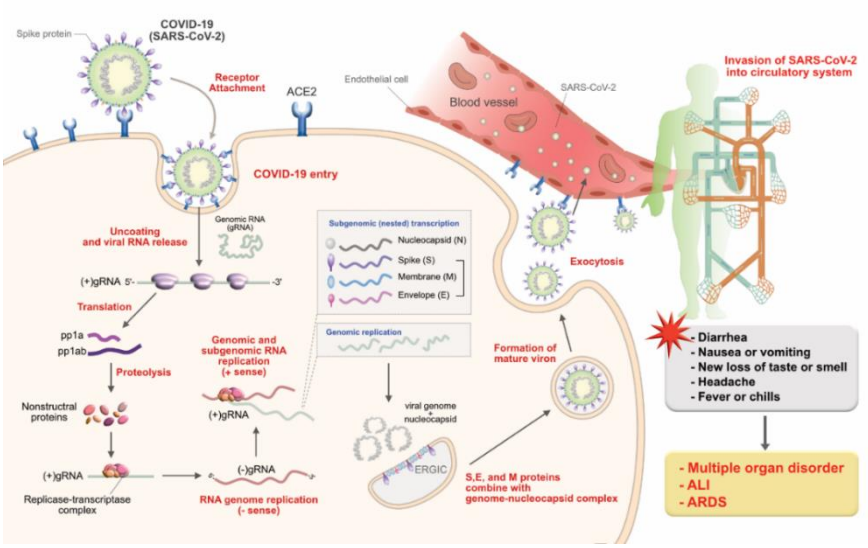


Fig. 2. Pathogenicity and life cycle of SARS-CoV-2 in host cells.

SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) cell receptors as a mechanism for entry into host cells, similar to SARS-CoV. Coronavirus entry depends on various cellular proteases, including cathepsins, transmembrane protease serine 2 (TMPRSS2), and human airway trypsin-like protease (HAT), which facilitate the splitting of the spike protein for penetration. After receptor binding, a conformational change in the spike protein aids viral envelope fusion with the cell membrane via the endosomal pathway. Viral RNA is then transcribed, with the viral mRNA directing protein synthesis. The virus replicates and assembles into new virions, which are then released into neighboring cells or blood vessels via exocytosis.

A huge protein complex is responsible for viral replication, and it is encoded for by a 20-kb replica gene (62). This replica complex is believed to comprise up to 16 viral subunits and a number of cellular proteins. Compared to other viruses, CoVs employ a variety of RNA-processing enzymes, including RNA-dependent RNA polymerase, RNA helicase, and protease, along with putative sequence-specific endoribonuclease, 3′-to-5′ exoribonuclease, 2’-O-ribose methyltransferase, and ADP ribose 10-phosphatase (63, 64). Proteins are assembled at the cell membrane, with genomic RNA incorporated as the mature particle buds from the internal cell membrane (65). CoV replication within host cells leads to a variety of effects, such as cellular

necrosis, lysis (66, 67), apoptosis (66), and cell fusion, forming syncytia (68). SARS-CoV, MERS-CoV, and SARS-CoV-2 exhibit high virulence, causing serious effects with a variety of symptoms, including fever, dry cough, myalgia, fatigue, and diarrhea. Severe disease progression results in ALI, ARDS, respiratory failure, heart failure, sepsis, and sudden cardiac arrest within a few days (51, 69). Pathological examinations of lungs from CoV patients show edema, proteinaceous exudates with globules, patchy inflammatory cellular infiltration, and bilateral diffuse alveolar damage with edema, pneumocyte desquamation, and moderate hyaline membrane formation (53, 70). These pathological features are commonly found in SARS-CoV, MERS-CoV, and SARS-CoV-2. Based on symptoms and disease severity, SARS-CoV-2 infections are usually markedly more severe than those associated with both SARS-CoV and MERS-CoV infections. Furthermore, the details of pathogenicity and life cycle of SARS-CoV-2 are described elsewhere (71-73).

4. SYNTHESIS, METABOLISM, AND BIOLOGICAL FUNCTIONS OF MELATONIN

Melatonin is an indolamine compound rhythmically secreted, according to the light and dark cycle, from the pineal gland into the cerebrospinal fluid and blood; it is metabolized in the liver and kidneys (74). Melatonin, which regulates circadian rhythms, is synthesized from its precursor, tryptophan, via tryptophan hydroxylase, arylalkylamine N-acetyltransferase (AA-NAT), and hydroxyindole-O-methyltransferase. It is also synthesized in other tissues, including the retina, bone marrow, and gastrointestinal tract, and is present in the bile (75, 76). Melatonin levels are lower in the day (light) and higher at night (dark). Following its synthesis, melatonin is first metabolized predominately in the liver, where it is hydroxylated by cytochrome P450 mono-oxygenases at position C6, prior to its conjugation with sulfate for excretion as 6-sulfatoxymelatonin (77). Further, melatonin is metabolized nonenzymatically in all cells, and extracellularly by free radicals and other oxidants (78). In brain and non-hepatic tissues, melatonin exists in the following form: *N*1-acetyl-*N*2-formyl-5-methoxykynuramine (AFMK). It exerts various functions, including sleep induction, resynchronization of biological rhythms, antioxidative, anti-inflammatory, and immunomodulatory activities, regulation of mitochondrial functions, and vasoregulation (Figure 3).

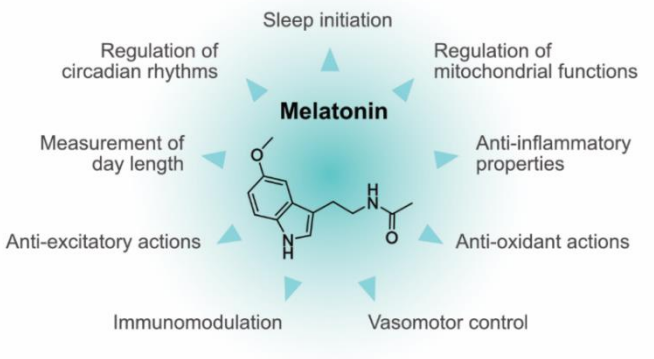


Fig. 3. A summary of the multiple functions of melatonin, many of which are applicable to its use as a potential treatment for COVID-19 disease.

Furthermore, melatonin regulates, and acts as an immunomodulatory, anti-oxidative, anti-inflammatory, and anti-apoptotic agent, by binding to receptors MT1, MT2, and MT3 (79). Additionally, it is able to bind to nuclear receptors RORα/RZR, which act as transcriptional

activators (80). Melatonin has various roles, including involvement in the cellular redox state, natural killer (NK) cell activity, and cytokine production, and has protective roles against chemotherapy and radiotherapy (81). Furthermore, it has protective effects against oxidative stress, resulting in inflammation, caused by ROS or reactive nitrogen species (RNS) (82). Melatonin not only has promising properties, such as cell permeability, the ability to cross both the blood–brain and placental barriers, and behavior as a free radical scavenger, but it also regulates various physiological processes, including the sleep wake cycle, pubertal development, and seasonal adaptation, through interaction with receptors MT1 and MT2 and intracellular proteins, such as quinone reductase 2, calmodulin, calreticulin, and tubulin (83). Alterations in melatonin receptor expression, as well as changes in endogenous melatonin production, was observed in various disorders, including sleep disorders, Alzheimer's and Parkinson's diseases, glaucoma, depressive disorder, breast and prostate cancers, hepatoma, and melanoma (84).

5. ANTI-INFLAMMATORY EFFECTS OF MELATONIN

While inflammation, marked by neutrophil infiltration, increased microvascular leakage, tissue swelling, and fever, is a major source of enhanced cytokine and chemokine production, melatonin causes a reduction in the production of both pro-inflammatory cytokines and chemokines. Anti-inflammatory effects of melatonin contribute to the modulation of the innate immune system. Although normal inflammation is necessary to assist tissue recovery following both infection- or non-infection-mediated injury, excessive inflammation can enhance or accelerate tissue injury. Melatonin protects carrageenan-induced local inflammation by scavenging peroxynitrite via its anti-inflammatory and antioxidative properties (85). Melatonin reduced innate immune responses, and thus, inflammation, by inhibiting the toll like receptor (TLR)4/ MAPK/NF-κB pathway in lipopolysaccharide (LPS)-treated neonatal rats (86). Melatonin suppressed the TLR2/MyD88/p-ERK pathway to reduce IL-2, IL-6, IL-10, IL-17, IFN-γ, and tumor necrosis factor (TFN)-α production, thus subsiding inflammation in *Helicobacter pylori*-infected mice (87). These effects were mediated by melatonin membrane receptors, MT1 and MT2, as anti-inflammatory effects were reduced by blocking these receptors (88, 89). Melatonin blocks the secondary inflammatory cytokine storm caused by damage-associated molecular patterns (DAMPs) of mitochondrial origin. This anti-inflammatory effect is the result of several sequential steps, including the reduction of cyclooxygenase-2 and nitric oxide (NO)-mediated activation of phagocytes and microglia (89, 90). Moreover, melatonin has been recently reported to shift macrophage polarization from proinflammatory type M1 to anti-inflammatory M2 (90). Although melatonin modulates a wide range of physiological functions, including pleiotropic effects on the immune system, at present, the precise mechanisms underlying its anti-inflammatory effects and how it regulates them, is not clear. A possible mechanism of MLT in numerous anti-inflammatory actions are shared by melatonin and SIRT1. SIRT1 supports anti-inflammatory actions of MLT upregulation (91). Melatonin downregulates sepsis-induced ALI through attenuation of lung injury and inflammation via SIRT1 (92). Through ALI infection models, a study demonstrated that melatonin was capable of anti-inflammatory activity and protective actions against ALI (93). It potentially suppresses NF-κB activation in ARDS, thus downregulating NF-κB activation in T cells and lung tissue (94, 95). Melatonin protects lungs from injury through Nrf2 stimulation (96). In mice subjected to chronic stress, the melatonin concentration sharply increased, exerting both anti-inflammatory and antioxidant effects (97). Melatonin treatment significantly reduced the Th1 CD4 lymphocyte population and increased the expression of the anti-inflammatory cytokine IL-10, as well as the

IL-10-producing CD4 T cell populations (98). Furthermore, it significantly inhibited inflammation *in situ*, reducing experimental autoimmune encephalomyelitis (EAE) severity in mice (99). Exogenous supplementation of melatonin suppresses NF-κB activation and induces anti-inflammatory effects in pineal microglia. Furthermore, melatonin treatment decreased inflammatory cytokine production by reducing NF-κB activation and increasing anti-inflammatory cytokine production in the lungs of influenza A virus-infected mice (100). Melatonin plays a critical role in the innate immune system and exhibits anti-inflammatory effects by modulating TLR signaling in brain ischemia, gastritis, and periodontitis disease models; it has been documented to possess anti-inflammatory actions via TLR4 signaling (6, 87, 101).

6. MELATONIN: ANTIOXIDANT AND INHIBITOR OF INFLAMMATORY AND IMMUNE RESPONSES

Antioxidative effects of melatonin are associated with anti-inflammatory effects. Melatonin could upregulate anti-oxidative enzymes and downregulate pro-oxidative enzymes, behaving as a free radical scavenger (102, 103). It is a powerful antioxidant and also has high bioavailability, penetrating both the blood-brain barrier and placenta (104, 105). Melatonin is not only synthesized by the pineal gland, but also by many other organs, including the gastrointestinal tract, retina, and leukocytes, both in the peripheral blood and bone marrow (8, 106). For instance, human lymphoid cells are a vital source of melatonin since both resting and phytohemagglutinin-stimulated human lymphocytes, which are not regulated by circadian cycles, synthesize five times more melatonin (107). Melatonin controls leukocyte function and contributes to inflammation control in tissues, acting as both an activator and inhibitor of inflammatory and immune responses (108, 109). An *in vitro* study suggested that melatonin administration enhances rat lymphocyte proliferation, increases the number of NK cells, stimulates the release of the pro-inflammatory cytokines IL-1 and TNF-α, enhances phagocytosis, and modulates apoptosis (8). Conversely, melatonin decreases upregulation of proinflammatory cytokines by inhibiting nuclear factor-kappa B (NF-kB) translocation to the nucleus (16). Melatonin prevents or reduces inflammation-derived activation of a variety of enzymes, including phospholipase A2, lipoxygenase, and cyclooxygenases (8). It is a powerful antioxidant, scavenging different types of free radicals and inducing the expression of various antioxidant enzymes, including superoxide dismutase, glutathione (GSH), catalase, GSH peroxidase, and GSH reductase. On the other hand, melatonin can reduce lipid peroxidation, which is involved in the pathogenesis of many diseases (110). Replication in viral infections constantly generates oxidized products. For example, a SARS-induced ALI model showed that oxidized low-density lipoprotein production activates innate immune responses through IL-6 overproduction from alveolar macrophages, via TLR4/NF-kB signaling, thus leading to ALI (111). TLR4 is an innate immune system receptor; it is also a therapeutic target for melatonin. Through TLR4 signaling, melatonin exhibited anti-inflammatory effects in various models, including brain ischemia, gastritis, and periodontitis disease (6, 87, 101).

Melatonin enhances mitochondrial biogenesis and increases mitochondrial GSH levels, leading to protection against free oxygen species and increasing electron transport chain efficiency in mitochondria (19, 112, 113). An experimental study demonstrated melatonin antioxidant activity using a mouse model of bronchiolitis, through respiratory syncytial virus (RSV) infection. RSV infection is characterized by a massive infiltration and activation of inflammatory cells in the airway, which subsequently produce ROS. RSV-infected mice exhibited elevated oxidative stress due to rises in NO, hydroxyl radicals (•OH), and malondialdehyde (MDA), which are associated

with decreases in GSH and superoxide dismutase (SOD) activities. Interestingly, pre-treating the animals with melatonin resulted in a marked reduction of severe disease-associated acute oxidative lung damage, such as in bronchiolitis and pneumonia (93, 114, 115). RSV infection stimulates the production of TNF-α, a prooxidant that can both stimulate the host inflammatory response and enhance NO synthesis. Increased NO production contributes to both airway inflammatory changes and airway dysfunction, while melatonin administration significantly inhibits NO production, eventually reducing pulmonary inflammation and airway hyperresponsiveness (116, 117). RSV infections induce production of proinflammatory and prooxidant cytokines through recognition of viral double-stranded RNA TLR produced during viral replication. Melatonin decreases TLR-mediated downstream gene expression in RSV-infected macrophages, as well as subsequent NF-kB-dependent gene expression (118). Taken together, these results conclude that melatonin can potentially inhibit RSV-induced injury to airway structures through oxidative stress inhibition and proinflammatory cytokine production; therefore, it may be a useful therapeutic agent in RSV-induced pulmonary disease. Melatonin inhibits various human pathogenic viruses, such as viral pneumonitis-induced excessive ROS generation (119). For instance, melatonin prevented inflammatory cell-induced responses, excessive inflammatory reaction, and oxidative damage, in infants, through suppression of NO, •OH, and MDA, and restoration of GSH and SOD levels in the lungs (93, 120). A mice study suggested that melatonin reduces RSV infection-induced oxidative stress proinflammatory cytokine production, such as TNF-α (93). Further, melatonin elicited various beneficial effects against RSV infections.

In addition, melatonin reduced the level of the proinflammatory cytokines IL-6, IL-8, and TNF-α in the tracheobronchial aspirate of newborns with respiratory distress syndrome (121). Oxidative stress-sensitive genes were upregulated in the peripheral blood mononuclear cells of SARS-CoV-2 human patients (122). Viral respiratory infections induce oxidative stress by elevating ROS and/or RNS levels (123). Oxidative stress induced the expression of PLA2G2D phospholipase in SARS-CoV (124).

7. MELATONIN: A POTENTIAL IMMUNOMODULATORY AGENT

The respiratory illness-causing viruses initially enter the body by first infecting respiratory epithelial cells; dendritic cells phagocytose the virus and present antigens to T cells, while cytotoxic CD8+ T cells produce and release pro-inflammatory cytokines which induce cell apoptosis (125). Excessive cytokine production leads to excessive recruitment of immune cells, resulting in uncontrollable epithelial damage, and generating a malicious circle for infection related ALI/ARDS (126). A clinical study suggested that COVID-19 infected patients showed reduced levels of neutrophils, lymphocytes, and CD8+ T cells in peripheral blood (127). Melatonin increased the immune response by improving the proliferation and maturation of NK cells, T and B lymphocytes, granulocytes, and monocytes in both bone marrow and other tissues (128). Melatonin administration increased antigen presentation in macrophages, with upregulation of complement receptor 3, MHC class I and class II, and CD4 antigens, was also observed (129). With consideration to ALI/ARDS, virus entry activated inflammation, triggered NLRP3 activation, and increased the rate of mortality, while melatonin treatment suppressed all of the aforementioned (130). Inflammasome NLRP3 activation is associated with infection-induced lung disease, including influenza A virus, syncytial virus, and bacteria (130-132). Further, in radiation-induced lung injury, allergic airway inflammation, and oxygen- and LPS-induced ALI models, melatonin inhibited inflammasome activation by reducing macrophage and neutrophil lung infiltration (102,

133). SARS-CoV-2 spread, infection, and cause of death leads to massive and prolonged stress, anxiety, and sleep deprivation, each of which leads to immune suppression. Chronic stress reduces the number and activity of protective immune cells, while stimulating immunosuppressive mechanisms and producing a pro-inflammatory response. Melatonin administration could be a useful sleep and immune response promoter, due to its potential immunomodulating effect (134, 135). As a immunomodulatory agent, melatonin increases ribavirin potency as an anti-influenza agent, with combinations of melatonin and ribavirin improving both replication inhibition outcomes and RSV infection (136). Animal models demonstrated that using melatonin as an antiviral immunostimulant prevents paralysis and death in mice infected with sublethal doses of the encephalomyocarditis virus; as well as reduces mortality in mice infected with encephalitis viruses (137, 138). Lack of sleep reduces the body's ability to respond to viral infections, which could be rectified with melatonin administration (139, 140). Therefore, melatonin stimulates several beneficial immunological responses, in turn, increasing immunity against viral diseases.

8. IMPACT OF MELATONIN ON INNATE AND ADAPTIVE IMMUNE RESPONSES

Immunity against any foreign substance is one of the most complex processes in organisms. For example, prokaryotes developed immune responses against viral infections (141). Clustered regularly interspaced short palindromic repeats (CRISPRs) are the basis for eliminating undesired genetic elements of foreign origin in prokaryotes and archaea (142, 143). CRISPR/Cas systems are considered as anti-viral immunity in prokaryotes (144-146). Eukaryotes contain a variety of defense mechanisms against both bacteria and viruses (147). Innate immune responses were evolutionarily conserved from prokaryotes to eukaryotes, including a number of secretory factors: from antimicrobial peptides to additional factors that attack pathogens in the extracellular space, or numerous regulatory molecules, which often bridge the innate and adaptive systems. Innate immune cells are considered first-line protectors; hence, they coordinate defense mechanisms and activate innate immunity to protect against multiple potential invasions; thus affording clearance of pathogens before they can enter the cell. Similarly, the adaptive immune system was also conserved from single to multicellular organisms, with highest complexity being found in vertebrates (148, 149). This system precisely targets specific pathogens using antibodies and cytotoxic T-cells. Both innate and adaptive immune systems function cooperatively to track, block, and finally destroy invading pathogens, including viruses, bacteria, pathogenic fungi, and parasitic animals. When foreign substances enter the body, both innate and adaptive immune systems are activated through a variety of immune cells, including macrophages and other monocytes, dendritic cells, basophils/mast cells, neutrophils, and NK cells (150). When viruses or bacteria enter the body, macrophages and neutrophils are activated to eliminate the pathogens via phagocytosis and chemical attacks. This recruitment occurs through secretion of cytokines, chemokines, and other signaling compounds, which organize the inflammatory response, thus accelerating both pathogen clearance and healing. Normal inflammation is essential for recovery following both infection- and non-infection-mediated injury. However, excessive inflammation causes injury to tissues via overreaction of the innate immune system to pathogen-associated molecular patterns (PAMPs), which is further magnified by DAMPs. Melatonin is able to control and regulate both PAMP- and DAMP-related innate immune system overreactions (151, 152). TLRs play a critical role in the innate immune system; recognizing structurally conserved molecules derived from pathogens to initiate inflammatory reactions in both immune and non-immune cells. During inflammation, but not during normal conditions, melatonin is able to

downregulate *TLR2*, *TLR4*, and *TLR9* expression, or inhibit their downstream pathways (153) Luo *et al*. (153) reported that melatonin suppresses the TLR2/MyD88/p-ERK pathway to reduce IL-2, IL-6, IL-10, IL-17, IFN-γ, and TFN-α production, thus subsiding inflammation in *H. pylori*-infected mice. Similarly, melatonin reduced the innate immune response by inhibiting the TLR4/ MAPK/NF-κB pathway through its membrane receptors MT1 and MT2, since its anti-inflammatory effects were reduced by blocking these receptors (88, 89). Through inhibition of the ERK1/2 and AKT pathways, melatonin suppresses TLR9-mediated innate immune responses without involving receptors MT1 and MT2 (154) .

Melatonin potentially eliminates NLRP3 inflammasome activation in septic mice via RORα, without involvement of melatonin receptors (155). Additionally, it suppresses NLRP3 inflammasome activation, airway leukocyte infiltration, goblet cell hyperplasia, and Th2 cytokine production (102)

Mitochondria play a major role in innate immune responses due to production of mitochondrial ROS, which stimulates the innate immune signaling cascade and intensifies inflammation induced by cytotoxic stimuli beyond microbial infection (156). Elevated melatonin levels in mitochondria protect their membrane potential by regulating the mitochondrial permeability transition pore (mPTP), thus preventing the release of mitochondrial contents (157, 158). Mitochondrial melatonin inhibits the secondary inflammatory cytokine storm caused by DAMPs. Melatonin can shift macrophage polarization from the proinflammatory type M1 to anti-inflammatory M2 (90).

Melatonin plays important roles in the adaptive immune system, regulating thymus specific cytokine production, decreasing the capacity for maturation and positive and negative selection of T lymphocytes, delaying and diminishing thymic involution, and promoting thymocyte regeneration (159, 160). Yu et al. reported that melatonin inhibits apoptosis during early B-cell development in mouse bone marrow, significantly promoting newly formed B cell survival and mediating humoral immunity [153]. Melatonin promotes both T-cell activation and differentiation, including Th17, Treg cells, and memory T-cells via activation of ERK1/2-C/EBPα (161). In addition, it positively regulates B lymphocyte activities, eventually increasing B-lymphocyte proliferation in birds and human tonsillar tissue (162, 163). Melatonin injections increase antibody titers and serum IgG levels (164). All these studies conclude that melatonin can serve as a hormone, paracrine, autocrine or tissue factor in functional immune system coordination. Therefore, melatonin deficiency significantly weakens the immune system, making it more prone to viral infections, such as COVID-19. Strong immune systems can defend against with foreign agents, thus providing longevity and health (165).

9. MELATONIN: IMMUNOSTIMULATORY AGENT

Melatonin's anti-inflammatory actions are attributed to its modulation of the innate immune system. Due to its anti-inflammatory and antioxidative properties, specifically its ability to scavenge peroxynitrite. melatonin protects against carrageenan-induced local inflammation (85). In addition, melatonin not only suppresses non-specific local, but also systemic, inflammation induced through zymosan treatment (166, 167). Melatonin blocks the secondary inflammatory cytokine storm caused by DAMPs of mitochondrial origin. Melatonin potentially suppresses NF-κB activation in ARDS and downregulates NF-κB activation in T cells and lung tissue (94, 95). It protects lungs from injury by stimulation of Nrf2 (96). The anti-inflammatory process involves sequential steps, including reduction of cyclooxygenase-2 and NO-mediated activation of phagocytes and microglia; as well as promotion of Nrf2 signaling through upregulation of sirtuin-

1 expression, a regulator with anti-inflammatory actions (94-96). In mice subjected to chronic stress, melatonin promoted both anti-inflammatory and antioxidant activities (97). In this experimental model, melatonin treatment significantly reduced Th1 CD4 lymphocyte populations, while increasing the expression of anti-inflammatory cytokine, IL-10, as well as IL-10-producing CD4 T cell populations (98). Exogenous supplementation of melatonin suppresses activation of NF-κB, inducing anti-inflammatory effects in microglia.

Through TRL4 signaling, melatonin plays a critical role in the innate immune system, activating anti-inflammatory responses and protecting against neural ischemia, gastritis, and periodontitis (87, 101, 168). TLR4 is both an innate immune system receptor and a therapeutic target for melatonin. Mice infected with RSV exhibited an elevation of oxidative stress, due to increases in NO, •OH, and MDA, which are associated with reductions in GSH and SOD activities. RSV infections stimulate production of TNF-α, a prooxidant which can both stimulate host inflammatory responses and enhance NO synthesis. Increased NO contributes to both changes in airway inflammation and dysfunction. RSV infections induce the production of both proinflammatory and prooxidant cytokines through recognition of viral double-stranded RNA TLRs produced during viral replication. Collectively, these results confirm that melatonin can potentially inhibit RSV-induced injury to airway structures, through inhibition of both oxidative stress and proinflammatory cytokine production. Thus, melatonin may likely be a useful therapeutic agent in RSV-induced pulmonary disease.

In infants, melatonin prevents inflammatory cell-induced reactions, as well as oxidative damage, by suppressing NO, •OH, and MDA generation, and restoring GSH and SOD levels in the lungs. A mouse study suggested that melatonin reduces RSV infection-induced oxidative stress resulting from proinflammatory cytokines, such as TNF-α (93). Additionally, in RSV infections melatonin lowers serum levels of lipid peroxidation products, and reduces plasma concentrations of IL-6, IL-8, TNF-α, and of nitrite/nitrate, thus increasing newborn survival (120, 121, 169). Moreover, melatonin reduces the level of proinflammatory cytokines, IL-6, IL-8, TNF-α, in newborn tracheobronchial aspirate associated with respiratory distress syndrome (121). In many experimental situations, melatonin increased the activity of SOD, glutathione peroxidase, reductase, and catalase (170).

Inflammasome NLRP3 activation is associated with lung disease caused by infection, including influenza A virus, syncytial virus, and bacteria (130, 131, 171). SARS-CoV-2 spread, infection, and cause of death leads to massive and prolonged stress, anxiety, and sleep deprivation, each of which leads to immune suppression. Chronic stress reduces the number and activity of protective immune cells, while stimulating immunosuppressive mechanisms and producing pro-inflammatory responses. Thus, melatonin stimulates several beneficial immunological responses, in turn, increasing the immune response against viral diseases.

10. MELATONIN: A NATURAL REMEDY TO REDUCE IMMUNOSUPPRESSION, INFLAMMATION, AND INFLAMMASOME ACTIVATION

The COVID-19 crisis has resulted in massive amounts of prolonged stress, anxiety, and sleep deprivation, leading to negative effects on the immune system and ultimately enhancing susceptibility to, and severity of, COVID-19; moreover, these individuals become more vulnerable to other diseases also. Stress, lack of sleep, and anxiety all lead to a suppression of immunity. Chronic stress promotes suppressive mechanisms while reducing the number and activity of protective immune cells; for example, both the number and activity of regulatory T-cells are

significantly reduced (135). Short and long term sleep deprivation causes profound changes in immunoresponsiveness, while short sleep durations may even result in hormetic effects (134). Melatonin secretion coincides with a peak in progenitor cell proliferation, resulting in their subsequent differentiation into granulocytes and macrophages (172-174); also increased melatonin levels at night with an increase in number of NK cells is a result of melatonin activation of T helper cells which produce several cytokines including IL-2, IL-6, IL-12 and interferon gamma (IFN-) (175). Sleeplessness results in an increased release of pro-inflammatory cytokines, including IL-1 beta, IL-6, and TNF-alpha, and reduced levels of anti-inflammatory IL-10 (176, 177).

Sleep deprivation is also a major factor in various metabolic diseases, such as cognitive, cardiovascular, metabolic, and other disorders possibly resulting from chronic inflammation (178, 179). Reduced levels of neutrophil phagocytosis, lowered levels of NADPH oxidase, and fewer CD4+ T cells, than in healthy volunteers, are consequences of sleeping less than 6 h per night in a 7-day period. These factors are critically important for anti-infective defense and a proper vaccination response. One report claimed that sleep deprived people immunized against the influenza A virus produced much lower levels of antibodies than those immunized without sleep deprivation (180). Similarly, lack of sleep caused lower antibody titers following immunization against the hepatitis A virus (181). A study utilizing a sleep deprived rat model demonstrated that pathogenic microorganisms could cause significant immune suppression, with long-term sleep deprivation leading to oxidative stress, and lower antioxidant enzyme activity in the rat hippocampus and brainstem (182-184). Production of melatonin is strongly associated with sleep, with individuals suffering from minimal sleep or chronic insomnia having lower levels of melatonin (185). Hence, experimental studies confirm that sleep is essential for an optimal immune response, while a lack of adequate sleep leads to stress and anxiety. Since melatonin favors good sleep, it may be an aid to reduce vulnerability to COVID-19.

Several previous studies have demonstrated that SARS-CoV-2 causes severe lung pathology via pyroptosis induction in macrophages and other immune cells, thereby leading to symptoms such as lymphopenia, which blocks effective immune responses to the virus (6, 186, 187). ORF8b-encoded viral protein directly interacts with inflammasome NLRP3, which activates the adaptor protein ASC and caspases 4, 5, and 11 (188). These changes lead to disruption of the cell membrane and release of inflammatory cell contents to the extracellular space, thus inducing pro-inflammatory cytokine secretion, including IL-1β and IL-18 (189, 190). Hence, pyroptosis inhibition, via NLRP3 activity, is vital; melatonin is a NLRP3 inflammasome inhibitor (133, 191). Several studies have demonstrated that melatonin is an effective pyroptosis inhibitor (133, 192-196). A systematic review and meta-analysis of clinical trial data revealed that, in a total of 22 randomized controlled trials, supplementary use of melatonin was associated with significant reductions in both TNF-α and IL-6 levels (197). These clinical data indicate that supplementary melatonin use may effectively reduce circulating cytokine levels, thus, potentially lowering pro-inflammatory cytokine levels in COVID-19 patients.

11. MELATONIN AMELIORATES VIRUS-INDUCED INFECTIONS

Melatonin is a potent regulator of immune function and a powerful free-radical scavenger (103). Melatonin reduced the death rate of Aleutian mink virus disease (AMVD) following subcutaneous implantation of melatonin-containing silastic capsules, which continuously release melatonin (198, 199). Additionally, melatonin ameliorated rabbit hemorrhagic disease virus (RHDV)-mediated fibroblast growth factor (FHF) by reducing the level of proinflammatory cytokines, endoplasmic

reticulum-mediated stress, and acute liver failure (200-202). During the recent Ebola outbreak, two research groups proposed a rationale for melatonin use as an alternative treatment for this deadly disease (203, 204). Melatonin battles Ebola virus disease (EVD)-induced infections by stimulating the immune system and its anti-inflammatory actions, and inhibiting platelet aggregation, thromboxane B2 production, and its free radical-scavenging effects (82, 91, 179, 205-208). Moreover, melatonin ameliorated EV-induced blood vessel endothelial damage, and vascular inflammation. Similarly, melatonin rescued endothelial vascular damage caused by bacterial LPS (209-211). Studies have also reported that melatonin inhibits pro-inflammatory cytokine secretion, reduces oxidative stress, and promotes the immune system (203, 204). Melatonin involvement in membrane barrier functions was documented via EVD-induced leakage of albumin, where it modulated the major Rho/ROCK pathway. This pathway is predominately involved in cytoskeletal maintenance and microfilament stabilization, which are both critical for normal membrane barrier physiology (212). These virus-related publications documented the ability of melatonin to suppress viral infections, suggesting its use as a potential treatment.

The inflammatory response is, first and foremost, the most important aspect of an influenza virus infection; it is essential in reducing viral load in the lungs (213). Combining melatonin and ribavirin significantly increased the survival rate of virus-infected mice, compared to that of mice treated with ribavirin alone (136). Melatonin treatment also significantly reduced Th1 CD4 lymphocyte populations, elevating the expression of anti-inflammatory cytokine IL-10, as well as IL-10-producing CD4 T cells populations (98). Melatonin also significantly inhibited inflammation, and reduced EAE severity in mice (99). Moreover, melatonin treatment of virus-infected Balb/c mice stimulated the expression of IL-10 and TGF-β, while inhibiting CD8 T cell-production of TNF-α (100). Melatonin may counteract viral infections by inducing the circadian gene *Bmal1*, which disinhibits the PDC responsible for conversion of pyruvate to acetyl-coenzyme A in the mitochondria. This change supports the tricarboxylic acid cycle and enhances both oxidative phosphorylation and ATP production (214), while reducing cytosolic aerobic glycolysis (215). Taken together, these findings suggest that melatonin may have therapeutic potential in both influenza-induced pneumonia and as an adjuvant treatment with anti-viral drugs.

12. IMPACT OF MELATONIN ON EBOLA VIRUS-INDUCED INFECTIONS

EV was first identified in 1976; it caused a serious outbreak between 2014 and 2016 in West Africa (216). According to WHO, EVD (*Zaire ebola*) is highly contagious, with an extremely high death rate; to date, there is still no clear medication for EVD. EVD has caused 11,000 deaths and resulted in the destabilization of various countries (217, 218). More than 50% of infected patients die from EVD. It infects through inadequately cooked flesh of infected animals, with several species carriers of the virus, including fruit bats, monkeys, and antelope. Further spread occurs when healthy individuals come into contact with soiled personal items from an infected individual or contaminated medical waste (219). Symptoms of EVD include abdominal pain, fever, diarrhea, muscle pain, and weakness (220). To reduce worldwide panic and potentially save lives, it was vital that medical researchers proposed alternative remedies, with positive effects, for this epidemic during the intervening period. During the Ebola outbreak, two research groups proposed a rationale for using melatonin as an alternative treatment for this deadly disease (204, 216). Due to the multifunctional aspects of melatonin, it was considered as alternative treatment choice. Melatonin could prevent EVD-induced weakening of the immune system, enhance blood coagulation, reduce excessive oxidative damage to cells, and prevent cellular and organ failure,

while inducing a marked inflammatory response (216, 221). Ebola infections can cause major hemorrhage shock due to blood vessel endothelium damage (222, 223). Conversely, melatonin battled EVD-induced infections by stimulating the immune system, and through anti-inflammatory effects, inhibition of platelet aggregation, thromboxane B2 production, and free radical scavenging (82, 91, 140, 179, 205, 207, 208). Melatonin ameliorated EVD-induced endothelial lining of blood vessels, endothelial damage, vascular inflammation, and endothelial dysfunction. Similarly, it rescued endothelial vascular damage caused by bacterial LPS (209-211). Studies reported that melatonin use inhibits pro-inflammatory cytokine secretion, reduces oxidative stress, and promotes the immune system (204, 216). Melatonin involvement in membrane barrier functions was documented via EVD-induced albumin leakage, where it modulated the major Rho/ROCK pathway; mainly involved in cytoskeleton maintenance and microfilament stabilization, both of which are critical for membrane barrier functions (212). Vascular permeability is an important factor for endothelial function, while increased levels of vascular permeability lead to various types of neurological diseases, including diabetic retinopathy, nephropathy, and vasculopathy. Melatonin may potentially inhibit vascular permeability in EVD-induced infections (224). EVD-induced vasculopathy is critically involved in hemorrhagic shock syndrome, often leading to death. Although, melatonin is not a viricidal agent, still it plays a major role in EVD and hemorrhagic shock syndrome (212, 225, 226). Taken together, these results demonstrate that melatonin is a useful natural molecule and a safe treatment.

13. ANTI-INFLUENZA POTENTIAL OF MELATONIN

Influenza, commonly known as flu, is an infectious disease caused by an RNA virus; it infects birds and mammals. In human society, influenza virus causes illness, death, and economic losses. Common symptoms of this disease are chills, fever, sore throat, muscle pain, severe headache, coughing, weakness, fatigue, and general discomfort. Influenza is a more severe disease than the common cold, caused by another virus (227). H1N1 influenza virus infections can lead to pneumonia and severe acute lung injuries. It spreads through viral particles, inducing various symptoms at onset, including dyspnea, hemoptysis, and pulmonary edema (228). Inflammatory responses to the virus are vital for its efficient clearance from the lungs (229). Lin et al. reported that melatonin treatment significantly reduced Th1 CD4 lymphocyte populations, while increasing the expression of anti-inflammatory cytokine IL-10, as well as IL-10-producing CD4 T cell populations (98). Further, melatonin treatment significantly inhibited inflammation, *in situ*, reducing experimental EAE severity in mice (99). Huang et al. found that melatonin treatment significantly increased the expression of IL-10 and TGF-β, while inhibiting CD8 T cell-production of TNF-α in virus-infected Balb/c mice (100). Interestingly, combining melatonin and ribavirin significantly increased the survival rate of virus-infected mice, compared to mice treated with ribavirin alone. Several studies have demonstrated that the aryl hydrocarbon receptor (AhR) is an important mediator, modulating antiviral immune responses to a variety of CoVs, including murine hepatitis virus (230). It is responsible for the expression of various genes, including TCDD inducible poly (ADP-ribose) polymerase (TiPARP), which is required for maximal CoV replication. The AhR also modulates macrophage and dendritic cell responses, including the levels of IL-1β, IL-10, and TNF-α (230). Overall, the AhR is a vital factor linking the initial “cytokine storm” and alterations in mitochondrial and immune cell function, including in the melatonergic pathway. All these studies suggest that melatonin possesses therapeutic potential in both influenza-induced pneumonia and as an adjuvant treatment with anti-viral drugs.

14. CURRENTLY AVAILABLE THERAPIES FOR COVS

To date, there are no available antiviral drugs or vaccines to counteract infection with COVID-19. Although an *in vitro* study suggested that the antiviral agent remdesivir and chloroquine may potentially control a COVID-19 infection, chloroquine's mechanism of action against viral infections remains elusive (231). One proposed mechanism of action may be the inhibition of pH-dependent steps in the replication of several viruses, specifically, it shows potent effects on SARS-CoV infection and spread (232, 233). Currently available broad-spectrum antiviral drugs, including lopinavir/ritonavir, neuraminidase inhibitors, peptide (EK1), and RNA synthesis inhibitors, could be alternative medications for COVID-19 infection (234). Remdesivir (GS-5734) exhibits broad-spectrum antiviral activity against several RNA viruses; interfering with the NSP12 polymerase (235). Holshue et al. (236) reported the first COVID-19 patient to be treated with remdesivir in the United States. Recent studies reported that combining either lopinavir, ritonavir or arbidol, with Shufeng Jiedu Capsule (SFJDC) significantly reduced pneumonia associated with COVID-19 (237), with lopinavir/ritonavir administration significantly reducing β-CoV viral loads in a COVID-19 patient in Korea (238). Thus, these combinations could be effective as both a prophylaxis and a therapy for human CoV (HCoV) infections, and have been positively tested in a rhesus macaque MERS-CoV model, yielding positive results (239, 240).

Synthetic DNA vaccines may provide multiple candidates for preclinical testing, resulting in scalable manufacturing of large quantities of drug products. For example, an engineered construct, INO-4800, showed significant effects on SARS-CoV and MERS-CoV (241). Administration of convalescent plasma or immunoglobulins reduced hospital stay duration and lowered the mortality rate in patients with SARS. Rajendran et al. (242) reported that, based on consolidated clinical data derived from five independent studies of 27 COVID-19 patients, convalescent plasma transfusion (CPT) may be an effective therapeutic option, with promising evidence for safety, improvement of clinical symptoms, and reduction of mortality. These drugs for COVID-19 treatment also use a protease called TMPRSS2 to complete the process (243, 244) (Figure. 4).

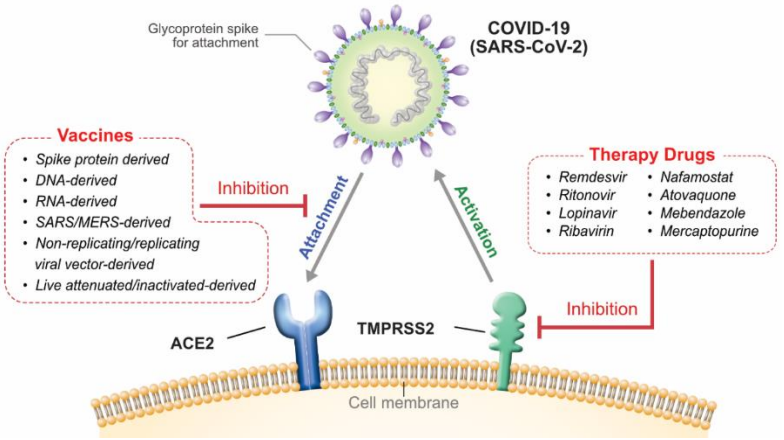


Fig. 4. Illustration of the connections of SARS-CoV-2 with the cell surface receptors as well as the potential inhibitors.

SARS-CoV2 causes pathogenicity when its spike proteins bind to angiotensin-converting enzyme 2 (ACE2), allowing entry into, and infection of, host cells. ACE2 activation may be caused via the cellular protease TMPRSS2. Majority of currently available therapies for coronaviruses prevent binding of SARS-CoV to ACE2 or prevention of cleavage process by TMPRSS2.

15. MELATONIN: ITS USE AS A REPURPOSED DRUG FOR COVS

Three highly pathogenic HCoVs, including SARS-CoV, MERS-CoV, and SARS-CoV-2, have emerged from animal reservoirs; leading to global epidemics with high morbidity and mortality rates (245). Zhou et al conducted a network proximity analyses of drug targets and HCoV–host interactions in the human interactome; for this study the authors used 16 potential anti-HCoVs repurposed drugs, including melatonin, mercaptopurine, and sirolimus (246). These candidate drugs were further validated via enrichment analyses of drug-gene signatures using HCoV-induced transcriptomics data in human cell lines. Among them, network-predicted evidence showed that melatonin indirectly targeted HCoV-associated proteins via human protein–protein interaction networks (Figure 5A).

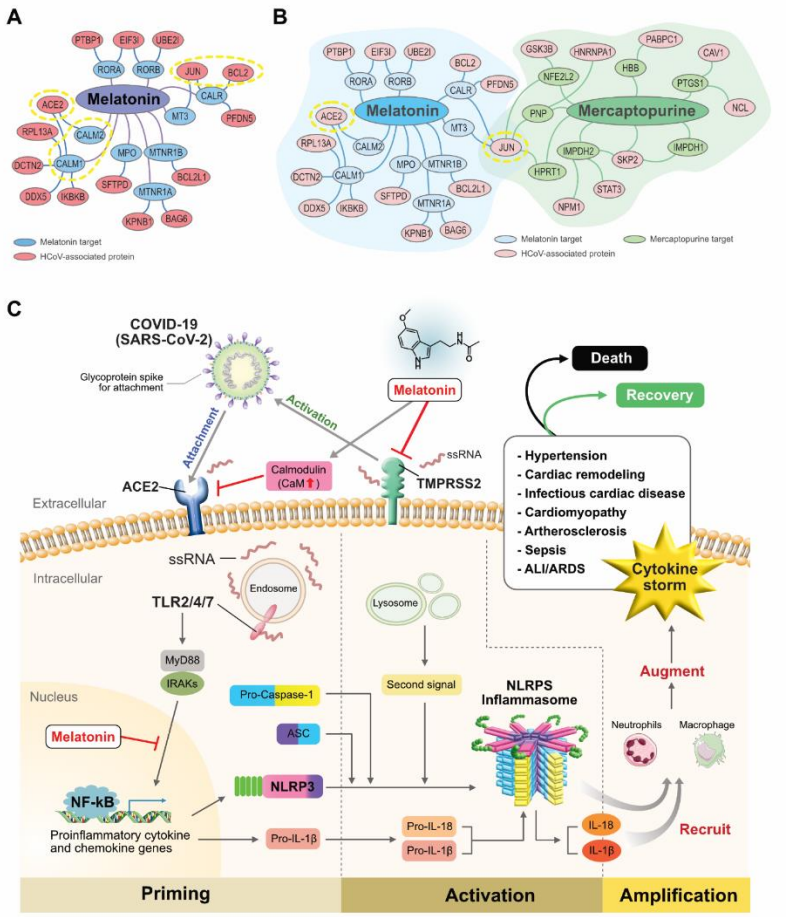


Fig. 5. Potential effects of melatonin on innate and adaptive immune system hyperresponsiveness induced by human coronaviruses (HCoVs).

Network-predicted evidence for melatonin (A) and melatonin and mercaptopurine combined (B) indicates that these drugs indirectly target HCoV-associated proteins via human protein–protein interaction networks [A- and B-derived figures are adapted from (246)]. (C) Application of melatonin as a potential antiviral vaccine adjuvant, or for preventing cytokine storm in COVID-19 patients, is also feasible. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) stimulates inflammasome activation, which can cause uncontrolled release of pro-inflammatory cytokines, leading to a cytokine storm and/or in some cases, death.

The gene set enrichment analysis (GSEA) score showed that melatonin achieved a GSEA score = 2 and Z = –1.72, indicating that these data sets met the criteria for melatonin application to SARS-CoV-2/COVID-19 management. Network data also suggested that mercaptopurine and melatonin may synergistically block c-Jun signaling by targeting multiple cellular targets, such as ACE2 and anti-inflammatory pathways. Furthermore, combining mercaptopurine and melatonin may offer a potential combination therapy for COVID-19/SARS-CoV-2 (Figure 5B).

Melatonin, as a multifunctional agent, plays crucial roles in various biological processes, offering a potential strategy for the management of viral infections via its anti-inflammatory and antioxidant capabilities (151, 203, 247-249). Viral infections often involve intense inflammatory processes, which increase oxidative stress and eventually cause negative effects on the function of many organs, a condition referred to as multiple organ failure; that commonly leads to death. Although melatonin is not directly involved in viral replication or transcription, it may indirectly attach to several HCoV cellular targets, including ACE2, BCL2L1, JUN, and IKBKB (82, 151, 203, 247-249). Notably, melatonin indirectly regulates ACE2 expression, a key entry receptor for HCoV viral infections, including COVID-19 (4). JUN is a key host protein involved in HCoV infectious bronchitis (250). Zhou *et al*. further demonstrated that mercaptopurine and melatonin may synergistically block c-Jun signaling by targeting multiple cellular targets (246). Combining mercaptopurine and melatonin may also synergistically impact papain like protease, ACE2, c-Jun signaling, and other anti-inflammatory pathways, thus providing a potential combination therapy for COVID-19. As shown in Figure 5C, melatonin reduces both the innate immune response and inflammation, by first inhibiting the TLR4/ MAPK/NF-κB pathway, and then suppressing the TLR2/MyD88/p-ERK pathway, thus reducing cytokine and chemokine production. These data are consistent with melatonin likely being a useful agent for reducing HCoV-induced cytokine storms.

16. THE ROLE OF MELATONIN AS A POTENTIAL ANTIVIRAL VACCINE ADJUVANT AND IMMUNE STIMULATOR

When given in combination with melatonin, DNA vaccine therapies may potentially decrease the viral load in COVID-19 patients. Subcutaneous administration of both the HPV-16 E7 DNA vaccine and melatonin in C57BL/6 mice has been shown to result in significantly greater HPV16 E7-specific CD8+ cytotoxicity, in addition to IFN-γ and TNF-α responses capable of reducing HPV-16 E7-expressing tumor volume; these changes were associated with enhanced survival time in TC-1 tumor bearing mice. Melatonin also lowered IL-10 and VEGF accumulation in the tumor microenvironment of vaccinated mice. These findings suggest that melatonin increases cancer vaccine efficiency, against HPV-associated tumors, in a dose dependent manner (251). Thus, melatonin may enhance the therapeutic efficacy of DNA vaccine potency in COVID-19 patients. SARS-CoV and MERS-CoV infected animals exhibit marked inflammatory and immune responses, through which a “cytokine storm” is activated, thereby elevating apoptosis frequency in both epithelial and endothelial cells; this contributes to vascular leakage, abnormal T cell and macrophage responses, induced ALI/ARDS, and sometimes death (252). In SARS-CoV-2 infected patients, lungs exhibit a cytokine storm, as evidenced by significant increases in levels of IL-1β, IFN-γ, IFN-inducible protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP-1), IL-4, and IL-10, compared to SARS-infected patients. These data show that significant difference exist between SARS (or MERS) and COVID-19, relative to the pathogenesis of the CoV (253). Together, all these findings indicate that inflammation is a major sign in COVID-19 patients, with excessive inflammation, depressed immune system function, and an activated cytokine storm

substantially contributing to COVID-19 pathogenesis. Imbalances between pro-inflammatory cytokines, chemokines, and anti-inflammatory molecules contribute to the development of this disease (254-256). The main reason for recommending the use of melatonin as an adjuvant is due to its unique properties, including anti-inflammatory, anti-oxidative, and immune enhancing. As noted above, several studies have previously documented melatonin's suppressive effects on viral infections, such as encephalitis; additionally, its use contributes to less viremia, lower acute oxidative lung injury, and less frequent paralysis and death.

Incorporating melatonin as an adjuvant treatment for deadly viral diseases, such as Ebola, influenza, SARS, MERS, and COVID-19, is not meant to preclude the use of conventional therapies, such as antiviral drugs. Relative to melatonin, other antiviral medications often have unwanted side effects. Melatonin is a natural, ancient, endogenously produced biocompatible molecule. Given its ubiquitous distribution in animals and plants, melatonin may be a suitable, versatile, and supportive therapeutic agent. The COVID-19 genome has 92.6% identity to SARS-related virus, SARS-CoV RaTG13, but it is less similar to SARS-CoV and MERS-CoV (257). Moreover, SARS-CoV-2 spike protein functional sites closely resemble those of pangolin CoVs (5, 258). SARS-CoV-2 has two different spike protein types, type L and type S, with a quantitative shift in favor of the S type having already occurred within a couple of months of the outbreak (257). These findings demonstrate the variability of viruses, which can generate new types and change their proportions within short periods of time. Variability and mutation frequency are a characteristic of animal and human influenza viruses (259, 260). Even if vaccines become available, changes at the target site may render them ineffective following short term of use, or some proportion of infected individuals may not be protected. Taking these factors into account, the need for a more generalized and less virus-specific therapy may be inevitable, especially to prevent lethal outcomes. Thus, it is seemingly necessary to identify an anti-inflammatory medication, not related to specific virus suppression, but rather focusing on providing adaptive immune system support. At this juncture, melatonin seems to be a suitable agent. The advantages of using melatonin as a supportive agent are not only related to its ability to promote the immune system in animals and humans, but also it seems to be effective against both various viral diseases and high-grade inflammation (120, 150).

In addition, a recent network-based bioinformatics study concluded that melatonin was a top candidate for viral disease treatment, with particular focus on COVID-19 (4). Finally, melatonin can be used either as an adjunct therapy, along with anti-viral medications, or as a regular systemic treatment for commencement at early signs of viral infection. Specific uses of melatonin may vary with patient health status and disease progression.

The National Institute of Allergy and Infectious Diseases (NIAID) in the United States of America (USA) conducted a safety and immunogenicity study on COVID-19 vaccine mRNA-1273, as a prophylactic treatment for COVID-19 infection. Next, it will be necessary to investigate adjuvant use to enhance vaccine efficacy during this crisis. Melatonin may be a good option. Effective vaccine responses depend on NK and CD4+ cells, as well as on cytokine production, which is enhanced by melatonin and influenced by patient age (261, 262). Melatonin, as a immunomodulatory molecule, is a feasible strategy (263). It prevents the development of paralysis and death in mice infected with sub-lethal doses of the encephalomyocarditis virus; as well as reduces mortality in mice infected with encephalitis viruses. It also improves immune responses after trauma-hemorrhage; considering these findings, melatonin may be useful as an antiviral immunostimulant (137, 138, 264). In addition, melatonin intake increases protection against other infections (139, 206). It can be used as an immunoadjuvant, which accelerates, prolongs, or

enhances antigen-specific immune responses by potentiating or modulating the immune system. ALI/ARDS animal model studies have shown that undesired or adverse effects following melatonin use are minimal (32, 265, 266). In humans, even when melatonin was administered to adults at a dose of one gram daily for 30 days, no toxicity was reported (267). Based on a recently published therapeutic algorithm, the use of large doses of melatonin alone, or in combination with hydroxychloroquine/chloroquine, could possibly resist COVID-19 infection (266). An even more recent study reported that combining vitamin D and melatonin may be an effective option for COVID-19 patients. Vitamin D and melatonin modulate and influence the immune system, limiting the oxidative response against COVID-19 infection (268). Collectively, these findings readily suggest that melatonin can be used as an adjuvant in viral therapy.

18. IMPACT OF MELATONIN ON COVID-19-INDUCED ENDOCRINE DYSFUNCTIONS

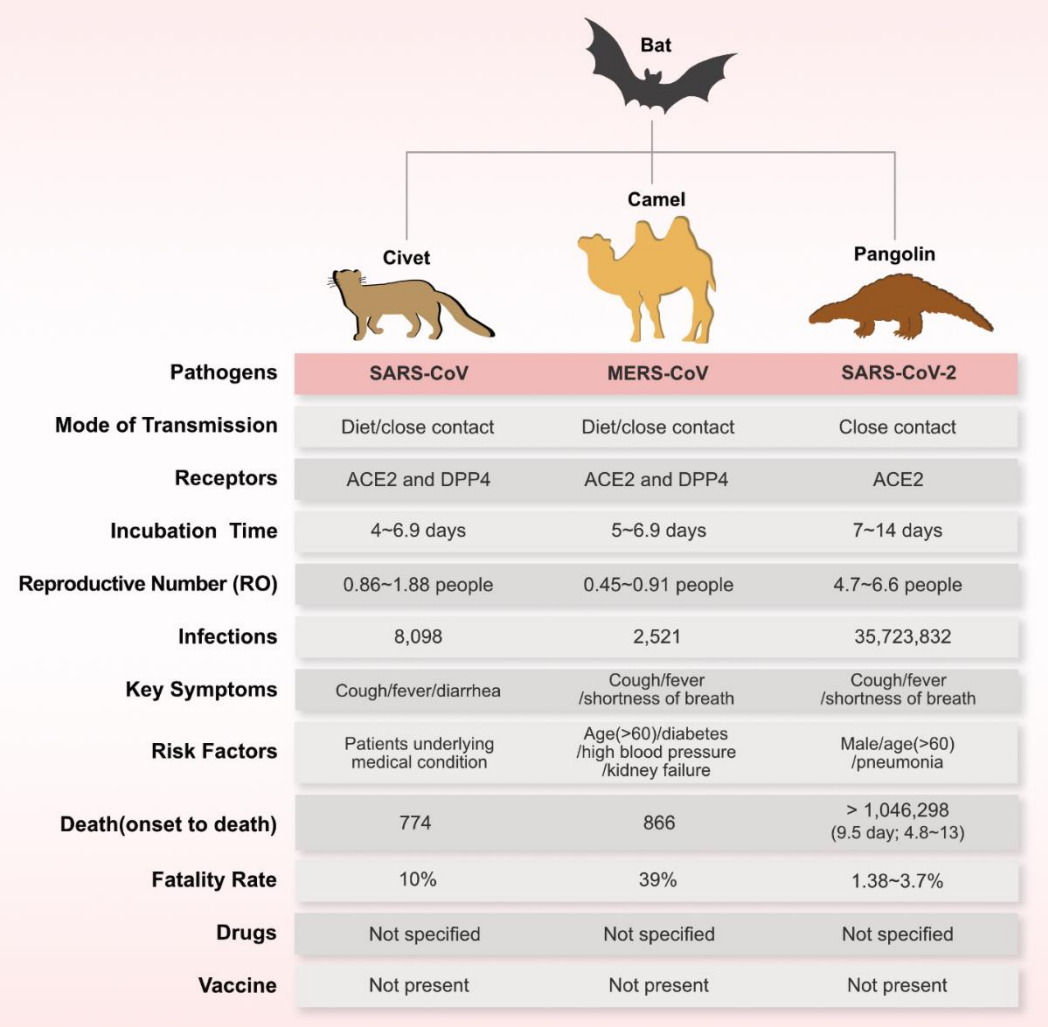
Type 2 diabetes mellitus (T2DM) is a susceptible risk factor for acquiring the SARS-CoV-2 infection, with T2DM and hypertension being identified as the most common comorbidities in other CoV infections, such as SARS, MERS, and SARS-CoV infections. Patients with SARS had significantly higher fasting plasma glucose levels, compared to patients with non-SARS pneumonia (269). Another study found that 'acute diabetes' in patients with SARS-CoV was due to pancreatic β-cell damage (270). These studies indicate that SARS-CoV could be a potential environmental trigger for T1DM development. The combination of CoV infection and T2DM triggers a dysregulated immune response, resulting in more aggravated and prolonged lung pathology (271). If patients with T2DM and metabolic syndromes contract COVID-19, they appear to be at a ten-times greater risk of death (272). As a result of cytokine storm, COVID-19-infected patients, with severe disease, are at a higher risk for multi-organ failure (273). SARS-CoV-2 enters human cells via an envelope spike glycoprotein, which is found on the surface of the virus, that binds to the ectoenzyme ACE2, and proteases such as TMPRSS2 to gain entry into the cell (243). ACE2 plays a significant role in converting angiotensin II to angiotensin 1–7; increasing ACE1 activity and inhibiting ACE2 leads to the activation of pro-inflammatory responses, stimulating aldosterone secretion via angiotensin 1 receptor (AT1R) or AT2R. As a consequence, these effects cause increased blood pressure, hypokalemia, and vascular permeability, eventually increasing the risk of respiratory distress syndrome (272). Conversely, angiotensin 1–7 acts on the Mas receptor pathway, which activates both anti-inflammatory and anti-fibrotic responses that would be favorable to recovery from COVID-19 (274). Taken together, these findings suggest that COVID-19 patients have an imbalance in the activation of these pathways. Increased levels of AT1R and AT2R activation causes T2DM, hypertension, and insulin-resistant states. ACE2 expression occurs not only in the lungs, but also in other endocrine organs, such as the pancreas, thyroid, testis, ovary, adrenal glands, and pituitary. ACE2 mRNA expression was significantly higher in the pancreas than in the lungs, with its expression being observed in both the exocrine pancreas and the islets. SARS-CoV and SARS-CoV-2 infections of surrounding exocrine pancreas caused the release of mediators, such as TNFα and IFN-γ, which may cause bystander β-cell death (275). COVID-19 may also lead to worsening insulin resistance in patients with pre-existing T2DM. A recent study reported that the level of serum total testosterone (T) was lower in 81 men with COVID-19, while that of serum luteinizing hormone (LH) was significantly higher, than those in 100 age-matched healthy men. Serum T:LH ratio was also significantly lower in COVID-19 patients, where it was negatively associated with disease severity (276). Elevated serum LH in

men with COVID-19 negates the possibility of hypothalamic–pituitary–testicular axis suppression, hinting toward primary Leydig cell damage. Patients with SARS exhibited noticeable destruction of both follicular and parafollicular cells in the thyroid, ultimately leading to low levels of calcitonin and causing osteonecrosis of the femoral head (277). Previous studies have reported that melatonin exerts anti-diabetic effects through controlling blood glucose in both animals and humans. Pinealectomized animals show lower expression of the glucose transporter type 4 (*GLUT4*) gene; as a result of low *GLUT4* expression, they develop glucose intolerance and insulin resistance. These conditions are alleviated through melatonin treatment (278, 279). Studies on mice further showed that genetic ablation of MT1 or MT2 affects glucose metabolism. MT1 knockout (KO) mice displayed systemic insulin resistance, marked by impaired skeletal muscle glucose uptake, adipose tissue glucose uptake, and significantly reduced liver insulin sensitivity (280). Collectively, these findings suggest that melatonin plays a significant role in regulating glucose metabolism, with disruptions in melatonin receptor signaling potentially contributing to T2DM pathogenesis. Based on experimental evidence, melatonin may have a potential role in glycemic control by both increasing insulin sensitivity and lowering fasting glucose. As such, it could be used to prevent COVID-19-induced diabetic complications.

19. CONCLUSIONS AND FUTURE PERSPECTIVES

In Figure 6, we summarize SARS-CoV-2 by comparing it to both SARS-CoV and MERS-CoVs. COVID-19 infections increase substantially in people aged 60 and older, as well as in males. Human-to-human transmission occurs primarily through respiratory tract droplets expelled from an infected person's cough or sneeze. The average incubation period has been calculated to range from 7 to 14 days, with each infected person able to infect 4.7–6.6 persons (281). SARS-CoV-2 can be a deadly virus due to its ability to hyperstimulate the innate immune response, including the inflammasome, which causes uncontrolled pro-inflammatory cytokine release, leading to a cytokine storm and severe, sometimes, irreversible damage to the respiratory epithelium. Further, SARS-CoV-2 powerfully activates the NLRP3 inflammasome. Melatonin's beneficial roles may be related to its ability to inhibit NLRP3 inflammasome-mediated IL-1β production. However, as it influences a variety of pathways, melatonin also has a much broader biological action. For example, melatonin modulates NF-kB upregulation and translocation, which is useful for counteracting COVID-19-associated hyper-inflammation. Considering this evidence, melatonin administration should be considered an alternative method to prevent the activation of the NLRP3 inflammasome and decrease NF-kB activation, and potentially inhibit COVID-19 replication. However, further research is necessary to prove its efficacy on the inhibition of NLRP3 inflammasome activation, IL-18 induction, and the "cytokine storm" at early stages of COVID-19 infection. Considering that melatonin modulates various aspects of the immune response, such as the production of IL-1β, IL-2, and TNF-α, inflammation, and oxidative stress, we propose that through changes in the melatonergic pathway, it may create links between the initial "cytokine storm" and alterations in mitochondrial and immune cell function.

Although melatonin is safe even at high doses (267), future work should focus on how best to administer it to decrease symptoms and fatalities resulting from COVID-19 infection. Whether melatonin specifically downregulates the circadian gene *Bmal1*, which disinhibits the PDC, should be investigated. Therefore, further studies are required to address whether SARS-CoV-2 downregulates the mitochondrial melatonergic pathway in immune cells, and other types of cells (214).



Pathogens	SARS-CoV	MERS-CoV	SARS-CoV-2
Mode of Transmission	Diet/close contact	Diet/close contact	Close contact
Receptors	ACE2 and DPP4	ACE2 and DPP4	ACE2
Incubation Time	4~6.9 days	5~6.9 days	7~14 days
Reproductive Number (RO)	0.86~1.88 people	0.45~0.91 people	4.7~6.6 people
Infections	8,098	2,521	35,723,832
Key Symptoms	Cough/fever/diarrhea	Cough/fever /shortness of breath	Cough/fever /shortness of breath
Risk Factors	Patients underlying medical condition	Age(>60)/diabetes /high blood pressure /kidney failure	Male/age(>60) /pneumonia
Death(onset to death)	774	866	> 1,046,298 (9.5 day; 4.8~13)
Fatality Rate	10%	39%	1.38~3.7%
Drugs	Not specified	Not specified	Not specified
Vaccine	Not present	Not present	Not present

Fig. 6. Comparison ofSARS-CoV-2 with both SARS-CoV and -CoV).

Compared to SARS-CoV (9.5%) and MERS-CoV (34.4%), SARS-CoV-2 appears to have a lower case-fatality rate (1.38–3.7%), but a significantly higher basic reproductive ratio (4.7–6.6) and incubation time (7–14 days). To date, no specific therapies have been demonstrated as effective against coronavirus disease (COVID-19), SARS-CoV, or MERS-CoV. Considering that melatonin has multifunctional effects, such as anti-inflammatory, anti-oxidative, anti-apoptotic, and neuroprotective capabilities, without any serious undesired side effects, it may justify the use of melatonin to attenuate the cytokine storm caused by SARS-CoV-2 infections.

Due to the rapid spread of, and high mortality rates associated with, COVID-19, waiting for vaccines is not practical; if no other treatment is identified soon, there will be an even greater loss of life and continued economic suppression. Viral infections can be minimized through the use of proven nutraceuticals and pharmaceuticals, which can lower the rates of infection and mortality. Melatonin is one such substance. Supplementation with melatonin may lower the rate of discernible infection signs, and directly or indirectly inhibit virus replication; thus, melatonin represents a useful molecule for treating viral infections. Further benefits of melatonin use were gained from its application in the management of previous viral infections, such as Ebola and

influenza, with this information potentially providing a road map for COVID-19 management. Overall, there are biomedical, financial, and moral reasons for developing and testing interventions that may immediately limit the impact of SARS-CoV-2 infections. Finally, network-based studies from COVID-19-affected countries, rapid and numerous screenings, and vaccine development have become the leading means for controlling the spread of COVID-19.

Abbreviations

CoVs; coronaviruses;
HCoVs; human coronaviruses
SARS-CoV-2; severe acute respiratory syndrome coronavirus 2
ALI; acute lung injury
ARDS; acute respiratory distress syndrome
COVID-19; coronavirus disease
MERS-CoV; middle east respiratory syndrome coronavirus
MT1; melatonin receptor 1
MT2; melatonin receptor 2
nm; nano meter
ORF1; open reading frame 1
RBD; receptor-binding domain
HE; hemagglutinin-esterase
IFNs; interferons
S; spike
E; envelope
M; membrane
N; nucleocapsid
nsps; non-structural proteins
ACE2; angiotensin converting enzyme 2
UTR; untranslated regions
TLR; toll-like receptor
MAPK; mitogen-activated protein kinase
NF-κB; nuclear factor kappa B
DAMPs; damage-associated molecular patterns
NO; nitric oxide
Nrf2; NF-E2-related factor 2
SIRT-1; nicotinamide adenosine dinucleotide (NAD)-dependent deacetylase
EAE; autoimmune encephalomyelitis
RSV; respiratory syncytial virus
OS; oxidative stress
OH; hydroxyl radical
MDA; malondialdehyde
GSH; glutathione
SOD; superoxide dismutases
TNF-α; tumour necrosis factor alpha
IL-Interleukin
ROS; reactive oxygen species

RNS; reactive nitrogen species
NADPH oxidase; nicotinamide adenine dinucleotide phosphate oxidase
NLR; nucleotide-binding domain leucine-rich repeat
SFJDC; shufeng jiedu capsule
CPT; convalescent plasma transfusion
AMVD; aleutian mink virus disease
RHD; rabbit hemorrhagic disease
EVD; ebola virus disease
PDC; pyruvate dehydrogenase complex
GSEA; gene set enrichment analysis
BCL2L1; Bcl-2-Llike protein 1
IKBKB; inhibitor of nuclear Factor kappa B kinase subunit beta
HPV; human papillomavirus
IFNγ; interferon gamma
VEGF; vascular endothelial growth factor
(IP-10); interferon-inducible protein 10
MCP-1; monocyte chemoattractant protein 1
NIAID; National Institute of Allergy and Infectious Diseases
RORs-RAR-related orphan receptors

ACKNOWLEDGEMENTS

Although we are the authors of this review, we would never have been able to complete it without the help of several people who have contributed to the research of coronaviruses and melatonin. We owe our gratitude to all these researchers who have made this review possible. We have cited as many references as permitted, and apologize to the authors of those publications that we have not cited due to reference limitations. We apologize to the other authors who have worked on these factors, but whom we have unintentionally overlooked.

This work was supported by a grant from the Science Research Center (2015R1A5A1009701) of the National Research Foundation of Korea and also supported by the KU-Research Professor Program of Konkuk University.

AUTHORSHIP

SG; conceptualization, performed all literature surveys, analyzed literature interpretation, and writing of the original manuscript; MHK; figure quality enhancement and reference arrangement; YC and RJR; manuscript writing and editing; JHK; conceptualization, writing, rearranging of figures. All authors agreed and approved this manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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Please cite this paper as:
Gurunathan, S., Kang, M.-H., Choi, Y., Reiter, R.J. and Kim, J.-H. 2021. Melatonin: A potential therapeutic agent against COVID-19. Melatonin Research. 4, 1 (Jan. 2021), 30-69. DOI:https://doi.org/https://doi.org/10.32794/mr11250081.