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Review Article

Melatonin interferes with COVID-19 at several distinct ROS-related steps

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ABSTRACT

Recent studies have shown a correlation between COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, and the distinct, exaggerated immune response titled "cytokine storm". This immune response leads to excessive production and accumulation of reactive oxygen species (ROS) that cause clinical signs characteristic of COVID-19 such as decreased oxygen saturation, alteration of hemoglobin properties, decreased nitric oxide (NO) bioavailability, vasoconstriction, elevated cytokines, cardiac and/or renal injury, enhanced D-dimer, leukocytosis, and an increased neutrophil to lymphocyte ratio. Particularly, neutrophil myeloperoxidase (MPO) is thought to be especially abundant and, as a result, contributes substantially to oxidative stress and the pathophysiology of COVID-19. Conversely, melatonin, a potent MPO inhibitor, has been noted for its anti-inflammatory, anti-oxidative, anti-apoptotic, and neuroprotective actions. Melatonin has been proposed as a safe therapeutic agent for COVID-19 recently, having been given with a US Food and Drug Administration emergency authorized cocktail, REGEN-COV2, for management of COVID-19 progression. This review distinctly highlights both how the destructive interactions of HOCl with tetrapyrrole rings may contribute to oxygen deficiency and hypoxia, vitamin B12 deficiency, NO deficiency, increased oxidative stress, and sleep disturbance, as well as how melatonin acts to prevent these events, thereby improving COVID-19 prognosis.

1. Introduction

In the severe stages of COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) infection, signs of hypoxemia accompanied by decreased response to oxygen therapy supplementation are present, regardless of the overall preservation of lung mechanics [1,2]. Certain reactions of the immune system during COVID-19 are associated with what has been characterized as a "cytokine storm" in which overwhelming amounts of reactive oxygen species (ROS) including superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), and peroxynitrite ($ONOO^-$), as seen in other inflammatory disorders, are produced (Fig. 1). Excessive activation of neutrophils caused by the overactive immune response generates myeloperoxidase (MPO) and contributes to the formation of neutrophil extracellular traps (NETs). NETs are mesh-like DNA fibers that are cast out from neutrophils in response to stimuli including microorganisms, microbial products, and chemokines [3]. The formation of NETs is highly dependent on, and most commonly triggered by, the protein kinase C (PKC) activator phorbol myristate acetate (PMA), a potent neutrophil stimulus that activates $O_2^{\bullet-}$ anion producing NADPH-oxidase [4]. PKC activation by PMA triggers the production of intragranular ROS. MPO activity through the generation of HOCl, along with other ROS, works to destroy assaulting pathogens, but may lead to tissue damage when HOCl is produced in excess [5–7] (Fig. 1). HOCl exists in approximate equilibrium with hypochlorite ion (^-OCl) at normal body pH 7.4 (pKa 7.59); however only the uncharged molecule can easily penetrate the cell membrane, most

Abbreviations: MPO, myeloperoxidase; ROS, reactive oxygen species; $O_2^{\bullet-}$, superoxide; H_2O_2, hydrogen peroxide; $^{\bullet}OH$, hydroxyl radical; $ONOO^-$, and peroxynitrite; NETs, neutrophil extracellular traps; HOCl, hypochlorous acid; NOS, nitric oxide synthase; NO, nitric oxide; Hb, hemoglobin; iNOS, inducible nitric oxide synthase; Fe, iron; H_4B, tetrahydrobiopterin; NOHA, N-hydroxyarginine; CAT, catalase; CNCl, cyanogen chloride; Co, cobalt; MMP, matrix metalloproteinase; HSA, human serum albumin.

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likely by passive diffusion through porins, proteins on the cell membrane that form channels [8,9]. MPO can be attached to extracellular NETs, be released at the plasma membrane due to inappropriate trafficking control, bind to the glycocalyx of several cell types and further increase neutrophil recruitment, or otherwise leak from the phagolysosome. All of these events can cause additional catalysis of HOCl [10]. In addition, the production of excessive amounts of ROS can interfere with the catalytic site of many hemoprotein model compounds, ultimately contributing to malfunction and/or destruction of hemoglobin and red blood cells, nitric oxide synthase (NOS), catalase, corrin rings such as in vitamin B12, zinc-Cys clusters, and sulfur-sulfur bonds, deficiencies of which are characteristic of COVID-19 [8,11–16]. HOCl might also modulate other important pathological mechanisms in COVID-19 including the release of free iron (Fe(II)) from HOCl mediated hemoprotein destruction, which reacts with HOCl directly or through the Fenton reaction generating $^{\bullet}OH$. Additionally, direct NO consumption by the near diffusion rate reaction of nitric oxide (NO) with $O_2^{\bullet-}$ generates $ONOO^-$ and leads to vasoconstriction [17,18]. Understanding these mechanisms will help the development of therapeutic strategies to combat SARS-COV-2 and other related inflammatory disorders.

Therapeutic treatment with melatonin has shown satisfactory outcomes in COVID-19 through potential preventive mechanisms, anticipated reduction of symptom severity, and possible reduction of immune pathology [19–21]. Ramlall et al., found that exposure to melatonin in intubated COVID-19 patients was associated with a more positive disease outcome [22]. It is thought that melatonin may not directly promote the host defense system against the virus but rather increase the tolerance of the host to the virus such as by suppression of inflammatory or apoptotic species e.g. nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) p65 or tumor necrosis factor-α (TNF-α) [19,20]. Furthermore, melatonin may be able to suppress cytokine storm effects on activation of monocyte/macrophage and inflammatory cytokine release, as well as inhibition of mammalian peroxidase chlorinating activity (Fig. 1) [21,23,24]. In addition, it has also been suggested that melatonin has preventive effects against SARS-CoV-2 infection by acting as an indirect inhibitor of angiotensin-converting enzyme 2 (ACE2) receptor coupling to SARS-CoV-2. Finally, this report highlights that melatonin can be an adjuvant to enhance the effectiveness of anti-SARS-CoV-2 vaccines [25]. The beneficial effects of melatonin in ameliorating COVID-19 symptoms have been attributed to its multiple roles as an antioxidant, anti-inflammatory, and immunoregulatory agent, as well as its ability to counteract circadian disruption, all of which allow melatonin to combat multiple diseases including metabolic syndrome, cancer, diabetes, infertility, and cardiovascular diseases [20,26] .

Melatonin is produced in the mitochondria of all organs in addition to its synthesis in the pineal gland; although, it is thought that the initial function was as an antioxidant during evolution with circadian rhythm effects evolving thereafter [27]. With the preventive benefits as stated above, melatonin is a stable and inexpensive over-the-counter drug, generally safe and effective for both short- and long-term use, displays a very high safety profile, can be easily synthesized when needed in large quantities, and can be easily self-administered [10,20,28–30]. Due to this convenience, Reiter et al., have suggested that melatonin treatment should be considered for prophylactic use, treatment alone, or treatment in combination with other drugs to fight SARS-CoV-2 infection [20]. The recommended pharmacological oral dose is 100–400 mg as an adjunct once a day immediately after contact with an infected SARS-CoV-2 individual or at the start of experiencing symptoms [20].

This review is an extension and continuation of our previous work [1,31], and differentiates from other reports on the subject, in that it discusses the ability of HOCl, the final product of MPO, to mediate the destruction of tetrapyrrole rings and the subsequent effects such as oxygen deficiency and hypoxia, vitamin B12 deficiency, NO deficiency, and the release of corresponding free metal. Also, this review unveils the mechanisms by which melatonin's actions as an antioxidant can be beneficial in preventing these effects thereby reducing dire outcomes through prevention of the pathophysiological consequences of SARS-CoV-2.

2. HOCl-associated hemoprotein heme destruction

As an important part of the first immune response, the activation of neutrophils results in the production of ROS; however, when ROS release is excessive due to excess stimulation of neutrophil activity, the result is often physiological damage such as cellular mitochondria poisoning, oxidative phosphorylation uncoupling, and lipid peroxidation. Of commonly appearing ROS, HOCl and H_2O_2 are long-lived compared to $O_2^{\bullet-}$ and $^{\bullet}OH$, and they can further generate $^{\bullet}OH$ upon reaction with free metal ions, a process that may be enhanced by $O_2^{\bullet-}$. HOCl can also directly react with $O_2^{\bullet-}$ to generate $^{\bullet}OH$ [32]. In turn, $^{\bullet}OH$ can

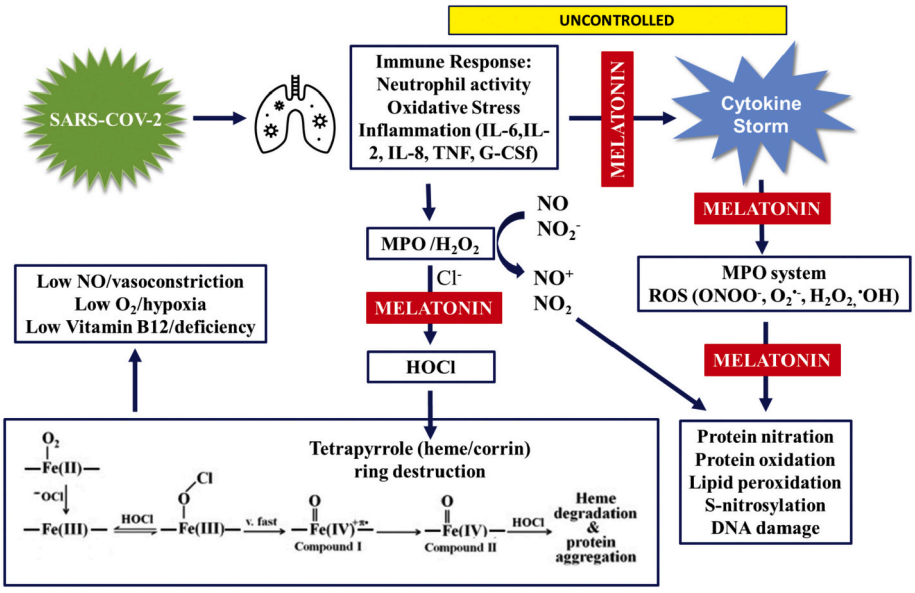

Fig. 1. The proposed pathway of SARS-CoV-2 infection associated with uncontrolled immune response and cytokine storm generating myeloperoxidase (MPO) and reactive oxygen spcies (ROS) resulting in decreased NO and vasoconstriction, decreased O_2 and hypoxia, and vitamin B12 deficiency through tetrapyrrole ring destruction mechanism, and the prevention by melatonin.

react with organic molecules at rates that approach diffusion-limited and contribute to hemoprotein heme modification [33].

MPO catalyzes H_2O_2-dependent oxidation of Cl^- through the formation of a ferryl π cation radical ($E - Fe(IV) = O^{\bullet+\pi}$) intermediate, compound I, to form HOCl [5,6]. Alternatively, Compound I can oxidize several organic and inorganic substrates (e.g., NO and nitrite (NO_2^-) to form nitrosonium cation (NO^+) and nitrogen dioxide (NO_2), respectively) during which the heme undergoes two sequential one e^- reduction steps producing compound II (MPO-Fe(IV) = O) and MPO-Fe(III), respectively [34,35]. Normal MPO levels range from 18 to 39 ng/ml in human plasma, with a significant increase up to 55 ng/ml and 287 ng/ml in inflammatory diseases, making MPO an efficient oxidative stress biomarker. HOCl accumulated by neutrophils have been reported at concentrations up to 25–50 mM/h, but is difficult to measure due to the amount of neutrophils present, MPO released, and H_2O_2 availability [10,36]. Enhanced levels of HOCl are sufficient to substantially damage biomolecules through processes such as thiol oxidation, chloramine formation, aromatic chlorination, and aldehyde generation [37]. Furthermore, HOCl can alter the biological function of hemoproteins through oxidation or destruction of heme (iron-protoporphyrin IX), an essential co-factor involved in multiple biological processes such as oxygen transport and storage, electron transfer, drug and steroid metabolism, signal transduction, and microRNA processing [1,11,12,15,38]. The destructive actions of accumulated HOCl, or other ROS, on hemoprotein heme moieties and other biomolecules could explain some features of COVID-19 and other similar respiratory disorders such as acute lung injury (ALI)/ acute respiratory distress syndrome (ARDS) [1,39–41]. These features include substantial hypoxemia not sufficiently explained by alveolar-parenchymal pathology, zinc deficiency, vitamin B12 deficiency, microvascular injury, thromboembolism, pulmonary hypertension, and damaged hemoglobin and red blood cell function accompanied by relative unresponsiveness to O_2 supplementation.

In the absence of an HOCl scavenger, there is a complex and interdependent relationship between levels of self-generated HOCl and MPO catalytic activity during steady-state catalysis. Enhanced self-generated HOCl in the milieu regulates MPO catalytic activity by heme degradation and subsequent free iron release, a process that is attenuated by HOCl scavengers (e.g. methionine) [38]. These studies clearly showed that self-generated HOCl may serve as a ligand for MPO, leading to catalytic inhibition and formation of a MPO–Fe(III)–OCl complex, which subsequently sets the stage for MPO heme destruction and free iron release. The direct reaction between HOCl with MPO–Fe(III) is fast and occurs with a second-order rate constant of $2 \times 10^8\ M^{-1}\ s^{-1}$ (pH 7) [42]. Key targets for HOCl-induced protein damage are various protein side chains, particularly those that are sulfur-containing. Rate constants reported by Davies and Pattison for reaction of HOCl and different amino acids range from $26\ M^{-1}\ s^{-1}$ to $3.6 \times 10^8\ M^{-1}\ s^{-1}$, a rate comparable to that of the direct reaction of HOCl with MPO [10,43,44]. Regardless of the variation in the rate constants among different amino acids, the final products for these reactions have been detected even for the lowest rate reaction, i.e. halogenated tyrosine has been proposed as a biomarker for detection of HOCl-induced protein damage [45]. Furthermore, an initial rate of $8 \times 10^3\ M^{-1}\ s^{-1}$ was found for the binding of HOCl to hemoglobin, further supporting the possibility of HOCl-mediated hemoglobin damage in a biological setting [12,43]. Similarly, an initial rate constant of $2 \times 10^3\ M^{-1}\ s^{-1}$ was found for the binding of HOCl to a vitamin B12 derivative [8,14]. In a previous study, 200 μM HOCl was found to react with and destroy the heme of hemoglobin in red blood cells [12]. This is consistent with recent work by Elahi et al., in which the harmful effect of COVID-19 on red blood cells is demonstrated [46].

As COVID-19 is known to be a disease of vascular endothelial inflammation, recruited neutrophils will likely be in relative proximity to passing erythrocytes [47]. HOCl and its adducts have been demonstrated to induce lysis of erythrocytes, exposing the erythrocytes' cytoplasmic hemoglobin to further oxidative damage [48–50]. Monochloramines derived from HOCl can also directly oxidize hemoglobin within erythrocytes without hemolysis [51]. Therefore, high concentrations of HOCl can mediate heme destruction of several hemoproteins and corrin ring compounds including hemoglobin and other related hemoprotein model compounds such as lactoperoxidase, myeloperoxidase, catalase, eosinophil peroxidase, and cobalamin derivatives, and more importantly can be prevented in the presence of 1:1 or 1:2 melatonin concentration [11,15,52–55].

Given the toxicity of overwhelming production of HOCl and downstream products such as free iron, HOCl is physiologically regulated. MPO protection against self-inactivation is a necessary process that prevents HOCl mediated MPO heme destruction, therefore allowing the enzyme to function at full capacity in generating HOCl [53]. One potential pathway of preventing MPO auto-inactivation is the rapid consumption of HOCl through rapid reaction with human serum albumin (HSA) and other proteins found in blood and other bodily fluids [56]. However, during COVID-19-associated inflammation, HSA and other HOCl scavengers suffer from decreased effectiveness due to competitive binding of SARS-COV-2, and HOCl production already exacerbated by inflammatory mediators is further unchecked [57,58]. However, HOCl consumption by reaction with these substances does not account for the complete loss of HOCl-dependent signaling and viral killing, suggesting that alternative pathways exist for HOCl depletion. In vitro studies demonstrate that HOCl can react with a verity of hemoprotein model compounds at various oxidation states (e.g., ferric, ferrous, ferrous-dioxy, and ferryl) [11,12,59]. Reactions of HOCl with hemoprotein intermediates that catalyze electron transfer reactions are another potential pathway for HOCl consumption.

Excessive HOCl levels may adversely affect both the oxidation state of the hemoglobin (Hb) heme iron as well as heme stability. For example, the initial step of the reaction of oxyHb, the main O_2 transporter, with HOCl involves oxidation of heme iron from ferrous to ferric state and subsequent release of O_2 (Fig. 1) [12]. Hb then enters a state that cannot bind oxygen, forming an Fe(III)-OCl complex by binding OCl^-. In the absence of a high O_2 concentration, the Fe(III)-OCl complex very quickly transitions to a hemoprotein ferryl π-cation complex, Compound I, via heterolytic cleavage of the O—Cl bond. Compound I is unstable and shortly decays into a ferryl complex, Compound II. In most cases of compounds with hemoprotein moieties, like in the case of hemoglobin, Compounds I and II populations depend on their formation and decay rates during steady state catalysis, and Compound II is known to be the most stable intermediate of hemoproteins. In vitro studies have shown that Compound II is also the predominant species formed prior to heme destruction, an irreversible step leading to depletion of the hemoprotein at hand [12].

Another family of essential enzymes that may be affected by increased HOCl production in COVID-19 are the three isoforms of NO synthases: inducible (iNOS), neuronal (nNOS), and endothelial (eNOS). These enzymes have many roles such as vascular smooth muscle control, inhibition of platelet aggregation, and vascular inflammation, whose dysfunction has been implicated in COVID-19 [47,60]. Reduction of NOS-Fe(III) by electrons provided by the reductase domain of the enzyme is the first step in the physiological reaction mechanism. Ferrous heme then combines with oxygen to form the corresponding Fe—O_2 complex. Further reduction of this intermediate by the cofactor H_4B leads to the formation of a ferric-peroxo complex (Fig. 1). Subsequent breakage of the O—O bond leads to the formation of highly valent ferryl porphyrin π- cation radical complex, compound I, which finally oxidizes the substrate, L-Arg, to form the intermediate N-hydroxyarginine (NOHA). Conversion of NOHA into citrulline and NO needs a second episode of oxygen activation, in which H_4B is thought to act as the electron provider. Destabilization of the NOS-Fe(II)-O_2 and/or Compound I intermediates by HOCl during NO synthase steady-state catalysis may disturb NO production, leading to vasoconstriction in COVID-19 patients [61–63]. Pathological HOCl levels may also diminish NO generation by mediating NOS dimer subunit dissociation and protein

unfolding through mechanisms that involve heme destruction and disturbance of the zinc-Cys cluster that controls H_4B binding and the enzyme stability (Camp et al., unpublished results).

Catalase (CAT) and mammalian peroxidases (MPO, EPO, and LPO) are other examples of heme-containing enzymes that can be affected by overproduction of HOCl [11,64]. These enzymes have been previously proposed to be involved in the etiology of various lung diseases ranging from cystic fibrosis to acute hypoxemic respiratory failure, and now possibly COVID-19 [65,66]. CAT and MPO have been shown to interact through diffusion of the substrate H_2O_2 and the product HOCl, regardless of the localization of CAT and MPO [64]. The interactions of OCl^- with both mammalian peroxidases and CAT include its ability to form the Fe(III)-OCl complex and subsequently generate compounds I and II (Fig. 1). Therefore, HOCl overproduction could lead to the imbalance of MPO and CAT through protein modification and/or heme destruction [64]. Inactivation of both enzymes leads to H_2O_2 accumulation, which, when combined with free iron, causes further oxidative damage to the host by generation of $^{\bullet}OH$. Therefore, the catalase/mammalian peroxidase heme destruction mediated by HOCl inhibits normal enzyme function, enhances iron (Fe) levels, and decreases levels of antioxidants. The severity of this process depends mainly on the bioavailability of HOCl [64].

Not only is heme destruction irreversible and depletes essential enzymes in the fight against SARS-CoV-2, but it also releases toxic products such as free iron which subsequently generates free radicals along with various protein aggregates. [1,12,15] In particular, free iron acts as the Fenton reagent, generating even more short-lived $^{\bullet}OH$ from longer-lived species such as HOCl and H_2O_2 [1,32]. Free iron can also damage blood vessels and increase vascular permeability through vasodilation [65–67]. This vasodilation not only leads to hypotension, but also metabolic acidosis. Iron overload may explain increases in the risk of ischemic cardiovascular events, accelerated thrombus formation, impaired vasoreactivity, and enhance the production of ROS in patients infected with SARS-CoV-2, found by some authors [1]. Therefore, in COVID-19, despite O_2/NO supplementation, the desired physiological response may be absent as essential heme-containing enzymes and proteins, such as Hb and NOS, have been disturbed biochemically through increases in HOCl and other ROS.

3. HOCl mediates corrin ring (vitamin B12) destruction

The association between COVID-19 and Vitamin B12 deficiency leading to worse outcomes of respiratory viral infections has been previously established [68]. Furthermore, COVID-19 and vitamin B12 deficiency have shared symptoms, such as increased oxidative stress, increased dehydrogenase, hyperhomocysteinemia, hypercoagulation, and vasoconstriction, particularly of the renal and pulmonary systems [69]. Consistent with this, recent investigation has shown that methylcobalamin supplements not only have the potential to reduce COVID-19-related organ damage, but also reduce other symptoms [68,69]. A clinical study has also shown reduced COVID-19 symptom severity in patients who received vitamin B12 (500 μg), vitamin D (1000 IU), and magnesium supplements, displaying significant reduction in the need for O_2 and intensive care support [69,70].

Using a variety of biochemical, physiological, and kinetic techniques, it has been shown that melatonin ceases HOCl-mediated vitamin B12 derivative corrin destruction [8,14]. Vitamin B12 deficiency has been associated with several conditions related to memory loss, immune system disorders, aging, heart disease, male infertility, diabetes, sleep disorders, depression, mental disorders, and inflammation [71]. Patients with one or more of these conditions might be more susceptible to severe SARS-CoV-2 infection [72]. In addition to ROS generated by the cytokine storm, SARS-CoV-2 infection may also interfere with vitamin B12 metabolism, therefore contributing to the pathogenesis of respiratory, gastrointestinal, and central nervous systems infections [69].

The two derivatives most commonly used for treatment of vitamin B12 deficiency are hydroxocobalamin and cyanocobalamin, with cyanocobalamin being preferred in the United States. However, outside of the U.S., such as in the United Kingdom, hydroxocobalamin is preferred due to its ability to firmly bind to plasma proteins allowing it to remain in the body longer [73]. Although the amount of scientific evidence is limited, the safety of cyanocobalamin supplementation for COVID-19 patients, versus other vitamin B12 derivatives, may be a concern. The biosynthesis of the B12 coenzyme from cyanocobalamin results in the release of CN^-, which could lead to acute cyanide poisoning, causing unwanted inflammation. Furthermore, when HOCl levels are high, HOCl-mediated reactions may cause the destruction of the corrin ring of vitamin B12, releasing cyanogen chloride (CNCl), free active cobalt (Co), and other corrin degradation products. Indeed, our previously published results suggest that the degradation of cyanocobalamin mediated by HOCl is largely modulated by the concentration of HOCl in the reaction milieu. Thus, any dysregulation of neutrophil/macrophage derived HOCl contributes to inflammation and, similarly, reduction in HOCl consumption could manifest as increased cyanocobalamin destruction and CNCl generation [14]. Therefore, it is of enormous therapeutic and pharmacologic importance to prevent HOCl-mediated damage, especially in chronic inflammation where a higher rate of infiltration of monocytes/macrophages over a longer period leads to pathologic alterations.

4. Mechanism of HOCl mediated tetrapyrrole ring destruction (Heme in hemoprotein and corrin ring in vitamin B12)

In hemoprotein, Fe is attached to the nitrogen of four pyrrole rings and a fifth proximal attachment to either the nitrogen of histidine such as in hemoglobin, catalase, and mammalian peroxidases or to the sulfur of cysteine, as seen in NOS and cytochrome P450. The sixth axial site of Fe can accommodate a variety of diatomic ligands such as O_2, NO, CO, CN^- and ^-OCl. The excessive production of ROS in the cytokine storm can result in modification of the heme prosthetic group inhibiting the protein function, whereas in vitamin B12 derivatives, the structure is based on a corrin ring containing four pyrrole rings attached to a center Co atom, distinguished by two directly attached pyrrole rings. The six coordination sites of Co in these compounds are the four pyrrole nitrogen atoms of the corrin ring, the nitrogen of the 5,6-dimethylbenzimidazole group at the lower (or α-) axial ligand, and naturally occurs with either a cyano-, hydroxo-, aquo-, methyl-, or adenosyl- group at the upper/β-axial ligand site [74]. Here, the overproduction of ROS and HOCl from the cytokine storm nonenzymatically mediates corrin ring destruction and the generation of free Co [14].

Pyrrole ring destruction by HOCl can occur as a result of direct attack on any of the carbon-methylene bridges between the rings, forming chlorinated adducts [12,14]. These chlorinated intermediates are unstable, quickly releasing Cl^- and decaying to an epoxide or aminal. The epoxide implements the buildup of a hydroxylated compound, with the $^{\bullet}OH$ group being connected to the carbon-methylene bridge of the porphyrin ring where the initial attack by HOCl takes place [12,14]. Attack by a second hydroxyl functional group creates vicinal diol, which can be split by either hemolytic cleavage or a transition metal mediated process (Fe, Co) by the formation of dioxetane intermediate through a heterolytic 2e- process; both processes result in a pair of carbonyl compounds. The aldehydes that are produced may be further oxidized by HOCl to create carboxylic acid through a mechanism previously described [75]. Cleavage of the C═C bond can take place at both the carbon-methylene bridge and the terminal C═C bond, leading to the generation of formaldehyde [12]. This single carbon aldehyde can be oxidized to formic acid by the electrophilic addition of HOCl. As recently reported, HOCl can alter the tetrapyrrole ring through a mechanism that involves disrupting the axial coordination of the Fe (hemoproteins) or Co (vitamin B12 derivatives) atom, causing ring destruction [8,14]. These alterations of the tetrapyrrole ring geometry might therefore make the ring a more eligible target to HOCl-attack and ring breakage,

which is associated with significant Fe/Co release.

5. Other effects of ROS

Generation of ROS and reactive nitrogen species (RNS) by the cytokine storm in COVID-19 can also result from increased matrix metalloproteinase (MMP) expression [76]. MMPs are zinc- and calcium-dependent enzymes associated with extracellular matrix remodeling that can also enhance activation of chemokines and cytokines from leukocytes [77,78]. In turn, generation of ROS from activated macrophages further activates and increases the expression of MMPs. Conversely, melatonin has been demonstrated to downregulate MMP expression, MMP protein level, and upregulate tissue inhibitors of MMPs [79,80]. These modulatory actions can prevent extracellular matrix remodeling associated with the inflammatory signaling molecules, especially interleukin-1β. ROS can also disrupt the function of metalloproteins by causing disturbance in their structural components and releasing free metals. Of note, HOCl can disturb the zinc-tetrathiolate cluster of iNOS leading to the irreversible release of zinc [81]. Other examples of ROS mediated metalloprotein disruption include the release of free iron from ferritin by $O_2^{\bullet-}$, as well as rapid inactivation of enzymes of the dehydratase-lyase family by $O_2^{\bullet-}$ followed by the oxidation of the iron-sulfur cluster releasing Fe(II) [82].

6. Melatonin is a potent inhibitor of MPO

Studies have shown the severity of disease is correlated with the level of inflammatory immune response caused from the pro-inflammatory cytokines released in the cytokine storm, with an exceptionally heightened response in those with more severe cases. Neutrophil activity, MPO activity, and ROS generation play an important role in the inflammatory immune response that contributes to the overwhelming production of HOCl; thus, MPO inhibition and elimination of unwanted ROS are undoubtedly important treatment targets in patients with COVID-19. It has been shown that triggering NET formation is dependent upon ROS formation and processing by MPO; therefore, inhibiting MPO could block the formation of NETs [4,83]. Melatonin is not only a reversible inhibitor and important regulator of MPO activity, but also plays an important role in detoxifying ROS. These phenomena position the indole as a powerful supplement to fight against early stages and severe SARS-CoV-2 infection. Melatonin-engaging therapies could reduce the stage of virus-associated pathology by controlling the host immune response to viral infection, mainly produced by alveolar macrophages and neutrophil MPO activity and unwanted ROS overproduction. Melatonin inhibits MPO chlorinating activity in a dual mechanism, which includes allosteric binding to the entrance of the MPO heme pocket and accelerating the formation and the decay of MPO compound II, which inhibits the chlorinating activity and slows down the peroxidation activity of the enzyme [23,24]. This allosteric binding to the MPO heme pocket entrance is enhanced by Cl^- binding to the halide-binding site, where HOCl is generated, and blocks H_2O_2 from the catalytic site. Thus, melatonin competes with H_2O_2 and switches the reaction from free to melatonin-bound enzyme (active to inactive form), and melatonin also competes with the co-substrate, Cl^-, and switches the reaction from a $2e^-$ to a $1e^-$ oxidation mechanism [23]. Studies have shown that rapid mixing of MPO preincubated with melatonin and Cl^- against the same volume of H_2O_2 solution caused immediate buildup of a transient intermediate, compound II, which then decays to MPO − Fe(III) through oxidation of another melatonin molecule, thereby closing the peroxidation cycle [23]. The ability of melatonin to compete with H_2O_2 and Cl^- on the active sites of MPO − Fe(III) and MPO compound I, respectively, is a key feature that drives the enzyme to alter its function to peroxidase activity. This mechanism of competitive inhibition has been shown to occur with tryptophan and several phenolic and aromatic amines [84].

More recently, kinetic evidence for an allosteric coupling among melatonin, the MPO entrance binding (regulatory) site, and the catalytic center that induces detectable inhibition of MPO has been provided [23,53]. Consequently, oxidation of melatonin is required before it diffuses from the binding site of the enzyme and restores the catalytic activity of MPO [23]. Thus, inactivation of MPO and its catalytic duration can be controlled effectively by melatonin supplementation. Indeed, when the melatonin concentration is less than twice the H_2O_2 concentration, H_2O_2 consumption proceeds in a slower and linear manner, and MPO decays rapidly to MPO − Fe(III) after melatonin oxidation. This behavior clearly demonstrates that MPO is capable of restoring its catalytic activity and rejoining the peroxidase cycle after melatonin exhaustion [23,53]. On the other hand, when the melatonin concentration is greater than twice the H_2O_2 concentration, the initial slow phase of H_2O_2 consumption remains at the same rate through the progression of the reaction and ceases when H_2O_2 is completely consumed [23,53]. The oxidation of melatonin is supported by the control studies that show no H_2O_2 consumption in the absence of MPO. When functioning as a 1e- substrate, melatonin competes with Cl^- and promotes alterations in substrate switching influencing the distribution of peroxidase intermediates capable of executing 1e- versus 2e- oxidation reactions. Understanding the circumstances that lead to MPO inhibition can provide important information and a general framework of understanding the role of MPO in certain diseases [85].

Computational molding of melatonin has shown the potential docking of melatonin to MPO-Fe(III), compound I, and compound II forms, although the crystal structure of MPO-melatonin complex remains unresolved. As melatonin is a relatively bulky molecule, the indole ring must dock parallel to the plane of the heme, with the side chain oriented towards the outside of the distal cavity [86]. Under these circumstances, the indole ring is close to the MPO D pyrrole with a distance average of the closest indole atom to the center of the D ring is ranging between 3.3 and 3.5 Å. Comparing MPO − Fe(III), compound I, and compound II modeling pointed that melatonin was deviated ~1 Å from the ferryl oxygen in compounds I and II. Therefore, the ability of melatonin to bind to MPO intermediates makes it an effective inhibitor of MPO activity.

7. Melatonin is a potent scavenger of ROS

ROS induced by viral infection are necessary for eliminating viruses phagocytosed by immune cells, and take part in signal transduction between various immune cells [87–89]. However, the inhibitory effects controlled by the immune system may be affected by aging and diseases (e.g. diabetes, cancer, and heart problems), causing some groups of individuals to be more vulnerable to SARS-CoV-2 infection [72]. Similarly, the inhibitory effects are also genetically controlled and thus may fluctuate with race and ethnicities [72,90]. Thus, the pathogenicity of new variants of SARS-CoV-2, varying immune response, imbalanced and uncontrolled ROS production associated with the cytokine storm, exuberant endothelial inflammatory reactions, vascular thrombosis, and bioavailability of enzymatic and non-enzymatic antioxidant machinery, in combination, may all become the determining factors that enable the virus to initiate infection [1].

Through melatonin supplementation, damaging conditions such as protein oxidation, lipid peroxidation, and DNA damage are diminished by reduction of ROS and free metal ion production [53,91–95]. Mass spectrometric and high-performance liquid chromatography studies have identified melatonin metabolites together with HOCl, intact tetrapyrrole rings, and hemoprotein, indicating that supplemented melatonin is likely consumed in exertion of its protective effects [54]. Furthermore, these melatonin metabolites are not known to have biological consequences, nor do they seem to effect MPO activity [23]. Other studies have highlighted melatonin's antioxidant ability in comparison with other known HOCl scavengers such as lycopene, methionine, taurine, cystine, cysteine, and uric acid [24,75,96].

Melatonin can also inhibit the activity of MMPs through binding of

melatonin to the active site, as shown in molecular docking simulations [97]. As previously explained, melatonin can further suppress deleterious effects of excessive MMPs by directly scavenging the ROS that increase MMP expression as well as protect against inflammatory lung pathologies, such as ARDS and COVID-19, through suppression of inflammation, oxidative stress, DNA damage, and cell apoptosis. [98,99].

8. Vitamin B12, melatonin, myeloperoxidase, and sleep

Vitamin B12 is essential for neural function, and a significant portion of the elderly population is deficient in this water-soluble vitamin [100]. Vitamin B12 accompanies melatonin as an antioxidant and a regulatory agent in circadian rhythm. By acting on the pineal gland, vitamin B12 takes part in rearrangement of the sleep-wake cycle through activation of the direct earlier release of melatonin at dark, as well as through causing melatonin to drift off faster by alerting the body to morning light [101]. Due to these properties, vitamin B12 derivatives (cyano-, hydroxo-, aquo-, methyl-, or adenosyl-) have been successfully used as therapeutic drugs for the treatment of serious sleep-wake disorders associated with COVID-19 patients [101].

Melatonin and peroxidase activities are apparently coupled through complex and interdependent pathways. It appears that the affinity of MPO for melatonin is very high under physiological conditions [23]. MPO may be acting as a chelator for melatonin and limiting its bioavailability and function. This may partly explain the harmful effects of sleep loss on various body functions. In addition, melatonin may have an important role in the inhibition of MPO activity in various tissues during inflammation. Hence, it is possible to speculate that increased inflammation and decreased immunity associated with lack of sleep are the result of a lack of inhibitory protective effects of melatonin on MPO during prolonged waking hours and chronic sleep loss. On the other hand, the pathogenesis of various disease processes can be very complicated and involves multiple inflammatory mediators and oxidizing agents. The biological consequences of melatonin−peroxidase interactions may have broad implications in the regulation of sleep, inflammation, infectious, cardiovascular events in vivo, and COVID-19.

9. Preserving lung mechanics through melatonin supplementation

Melatonin potentially has several other benefits against COVID-19 in addition to what has already been listed. As discussed by Zhang et al., in the case of COVID-19, there is high risk of developing ALI/ARDS and other complications such as respiratory failure, heart failure, sepsis, and even sudden cardiac arrest, a progression similar to that of SARS- and MERS- induced pneumonia [21]. Melatonin can act against several elements of ALI/ARDS processes. It mediates activity of sirtuins such as sirtuin-1, which can downregulate transition of macrophages towards pro-inflammation through inhibition of high mobility group box chromosomal protein 1 and participate in other antiviral processes [102–104]. Melatonin can also suppress the activation of NF-κB, a pro-inflammatory mediator in ALI/ARDS, both in T cells and lung tissue [105,106]. Nuclear factor erythroid 2-related factor 2 (NRF2), a pulmonoprotective factor, has been shown to be enhanced by melatonin and has been postulated to be involved in CoV-associated ALI/ARDS. Melatonin also upregulates anti-oxidative enzymes and downregulates pro-oxidative enzymes and has been shown to directly enhance the immune response through improvement of proliferation and maturation of natural killing cells, T and B lymphocytes, granulocytes, and monocytes [107]. Melatonin has also been shown to decrease the level of circulating cytokines [21]. These anti-inflammatory and pulmonoprotective effects of melatonin further suggest its possible suitability for treatment of COVID-19.

10. Conclusion

With the discovery of multiple COVID-19 variants spreading around the world, the question remaining is can the current vaccines keep up, or will they become less effective against SARS-CoV-2 mutations? There is a possibility that the cost and time needed to develop a vaccine targeted at new strains may not be met before another spike occurs. In this case, recognition of treatment options is of utmost importance. Melatonin is particularly notable, as its supplementation is safe for use with other treatments, as it has been previously given to COVID-19 patients in combination with the US Food and Drug Administration emergency authorized cocktail, REGEN-COV2 [20,21].

Many of the known clinical signs of COVID-19 could potentially be associated with defects in the function of several key tetrapyrrole and heme proteins, notably hemoglobin, cyanocobalamin, and NO synthases. Here, we have outlined the destructive effects of ROS, especially HOCl, on these biomolecules. As one of the major sources of HOCl is through overactivity of MPO provoked by the cytokine storm of COVID-19, inhibition of MPO and/or elimination of HOCl may play a beneficial role in diverse biological processes by reducing the metal release mediated by HOCl and associated increases in other ROS. Related studies from our lab have shown that MPO and its effects can be inhibited at three points: 1) through heme reduction that causes collapse or narrowing in heme pocket geometry that prevents the access of the substrate to the catalytic site of the enzyme (e.g. ascorbate) [108]; 2) switching the MPO catalytic cycle from peroxidation to catalase-like activity (e.g. melatonin, tryptophan, tryptophan analogs) [23,84,109]; or 3) direct scavenging of HOCl (e.g. lycopene) [75].

The ability of melatonin to act as an antioxidant, anti-inflammatory, and immunoregulatory agent allows the unique opportunity for its use in a variety of therapeutic approaches, and it has shown promising benefits in cancer, diabetes, infertility, and inflammatory diseases. The outstanding benefits of melatonin provide an accessible, relatively inexpensive, and safe treatment that may ease COVID-19 symptoms commonly seen with the cytokine storm and overactive immune response, leading to improved patient outcomes and reduced suffering. In addition to use alone, promising evidence is emerging to establish melatonin as a therapeutic treatment that could potentially be used in combination with current antiviral agents. Further clinical and experimental studies are required to confirm the applied benefits of melatonin supplementation as a therapeutic agent for treatment of patients infected with SARS-COV2.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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