Minireview *Highlight article*

Potential therapeutic effects of Ivermectin in COVID-19

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Impact Statement

Ivermectin is naturally derived from the fermentation of *Streptomyces avermitilis*. Ivermectin is known as a drug with a wide range of pharmacological properties, including antimicrobial, anticancer, and antiviral effects. Ivermectin has a record of safety in human use, with the total distributed doses in one-third of the world population, in the past 30 years. Of note, there is convincing evidence showing the therapeutic potential of Ivermectin for treating COVID-19. Several clinical trials have demonstrated ameliorating effect of Ivermectin on the symptoms of the disease, and a growing number of ongoing clinical trials are evaluating its therapeutic efficacy and safety in patients with various stages of the disease. Moreover, mechanistic studies have indicated that Ivermectin can selectively inhibit molecular targets involved in the replication and infection of the SARS-CoV-2 virus.

Abstract

COVID-19 is a critical pandemic that affected communities around the world, and there is currently no specific drug treatment for it. The virus enters the human cells via spikes and induces cytokine production and finally arrests the cell cycle. Ivermectin shows therapeutic potential for treating COVID-19 infection based on *in vitro* studies. Docking studies have shown a strong affinity between Ivermectin and some virulence factors of COVID-19. Notably, clinical evidence has demonstrated that Ivermectin with usual doses is effective by both the prophylactic and therapeutic approaches in all phases of the disease. Ivermectin inhibits both the adhesion and replication of the virus. Local therapy of the lung with Ivermectin or combination therapy may get better results and decrease the dose of the drug.

Keywords: Ivermectin, COVID-19, pandemics, SARS-CoV-2, hypoxia-inducible factor-alpha, importin, inflammation, lung

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COVID-19 and pathogenesis

The lack of specific treatment for the COVID-19 pandemic necessitates the development of effective drugs.[1,2] The study on its pathogenesis helps drug or vaccine development against this disease.[3] The virus enters human cells via binding of the spike protein to the angiotensin-converting enzyme-2 receptor (ACE2) in many tissues, especially epithelial cells of human alveoli.[4] After binding, essential changes happen in the spike protein by FURIN, cathepsin, or transmembrane serine protease 2, which are essential events for connecting to the membrane and promoting infection.[5–8] FURIN cleavage site probably has an important role in SARS-CoV-2 virulence, because the mutant lacking the FURIN cleavage site shows a reduced replication in Calu3 cells and an attenuated disease burden.[9,10] Spike protein has two distinct structures, before and after fusion, with specific features, showing a protective role against immune response.[11] Structural studies on the SARS-CoV-2 spike proteins indicate similarity with the FURIN segment of the sodium channel in human epithelial cells,[12] which makes a competition between them. Molecular studies on SARS-CoV-2 and the host indicate the effect of phosphorylation of virus proteins on the activity of kinases and host cell growth factor receptors. The virus finally induces cytokine production and silences CDK1/2/5 (cyclin-dependent kinase), resulting in cell cycle arrest.[13]

COVID-19 infection in the lungs has three main phases: the first or early infection phase is viral replication with somewhat mild symptoms; the second or pulmonary phase is determined by adaptive immunity stimulation and exacerbation of respiratory symptoms; and, in some cases, the third and last phase is a hyperinflammatory state or phase. According to the phase of infection, clinical symptoms range from mild such as fever, fatigue, cough, or myalgia, and sore throat or headache to acute respiratory distress syndromes such as hypoxemia and shortness of breath to shock and failure of organs.[14]

Ivermectin

Avermectins are naturally derived from the fermentation of *Streptomyces avermitilis*. Avermectin-derived drugs include Ivermectin, Selamectin, Duramectin, Eprinomectin, and Abamectin. The discovery of Ivermectin led to the Nobel Prize in Physiology or Medicine in 2015.[15–17] Ivermectin is known as a drug with a wide range of pharmacological properties, including antimicrobial, anticancer, and antiviral effects.[18–22] Ivermectin is mostly used for onchocerciasis treatment and can be effective against *Enterobius vermicularis*, *Ascaris lumbricoides*, *Trichuris trichiura*, and *Strongyloides stercoralis*.[23–26] Besides, recent studies have shown the antiviral effects of Ivermectin on SARS-CoV-2, HIV-1 (human immunodeficiency virus), Avian Influenza type A, Newcastle disease, yellow fever, and West Nile virus.[27] Ivermectin has a record of safety in human use, with total distributed doses in one-third of the world population, in the past 30 years.[28] In a study, treatment with higher FDA-approved doses of Ivermectin (200 μg/kg) was well-tolerated even with 2000 μg/kg, which is 10 times more than the FDA-approved dose, and only moderate central nervous system toxicity was seen.[29–31] Ivermectin should not be used during pregnancy, because safety in pregnancy has not been established.[32]

Ivermectin shows therapeutic potential for treating COVID-19 infection

Recent research has shown the strong antiviral properties of Ivermectin by inhibiting the replication of some RNA viruses *in vitro*.[33,34] Ivermectin is selectively concentrated in the pulmonary tissue, about 3 times more than the plasma concentration, and remains in the pulmonary tissue for a long time.[35] There is convincing evidence showing the therapeutic potential of Ivermectin for treating COVID-19. Several clinical trials have demonstrated the ameliorating effects of Ivermectin on the symptoms of the disease, and a growing number of ongoing clinical trials are evaluating its therapeutic efficacy and safety in patients with various stages of the disease. Moreover, mechanistic studies have indicated that Ivermectin can selectively inhibit molecular targets involved in the replication and infection of this virus. Here, we aimed to review published studies, evaluating the effect of Ivermectin on COVID-19 virulence, together with the underlying molecular mechanisms.

In vitro studies

Ivermectin has shown an inhibitory effect on the growth of some RNA viruses, and DNA viruses such as Pseudorabies Virus, SARS-CoV-2, HIV-1, Avian Influenza type A, Newcastle disease, yellow fever, and West Nile virus,[27,36–41] An *in vitro* study showed that the addition of a single dose of Ivermectin (5 μM) to Vero-hSLAM cells 2 h post-infection with Australian SARS-CoV-2 could reduce viral RNA about 5000-fold at 48 h. The IC_{50} value of the drug at 48 h postinfection, was found 2.2–2.8 μM and no cytotoxicity was observed.[42] This finding warrants further study for the possible benefits of Ivermectin in humans, although it has not been tested in any pulmonary cell lines, which are determinants for SARS-CoV-2 in humans.[28]

Clinical evidence

Ivermectin has been reported to be effective by both prophylactic and therapeutic approaches in all phases of the disease from mild to severe.[35,43] Primary evidence recommends that treatment with Ivermectin in patients with COVID-19 can reduce mortality, especially in cases that need oxygen or mechanical ventilation.[44]

The results from clinical trials that used Ivermectin in a dose ranging from 200 to 1200 μg/kg for three to seven days showed a viral load reduction and promising observations of the disease symptoms.[32] The single standard dose of Ivermectin (9 mg) has shown cases of rapid clinical resolution in severe hospitalized patients of COVID-19.[45,46] Other doses, 12 mg/day for —one to two days in patients with body weight < 75 kg, as well as 600 or 300 μg/kg/day for five days, are now under phases II and III of clinical trials for COVID-19.[47] However, these dose plans are more than the approved dose of Ivermectin that is, a single dose (200 μg/kg) for the treatment of strongyloidiasis, and thus ignores consistent exposure.[48] A pilot clinical study conducted on hospitalized adult patients with mild to moderate SARS-CoV-2 infection revealed that add-on therapy of Ivermectin to hydroxychloroquine/azithromycin exerted better effectiveness, shorter hospital stay, and relatively safe compared with controls. Notably, the average time to stay in the hospital was significantly lower in patients received a single oral dose of Ivermectin (200 μg/kg) as add on therapy compared with the controls (7.62 ± 2.75 versus 13.22 ± 5.90 days). Notably, two patients died in the control group, none in the Ivermectin group.[49] The other clinical trial indicated that one or more doses of Ivermectin (200 μg/kg) in addition to usual clinical care could significantly reduce the mortality rate in patients with SARS-CoV-2 infection when compared with the controls (15% versus 25.2%).[50] Moreover, the use of Ivermectin 150 μg/kg in 52 patients after mechanical ventilation showed a potential decrease in hospitalization length and survival compared with 1918 patients who were treated conventionally.[51] In another clinical study, patients (older than 40 years) at emergency rooms were received different medications, including Ivermectin, azithromycin, and oseltamivir; unfortunately, such treatments could not considerably reduce hospitalization risk.[52] Notably, the results of a double-blind, randomized clinical trial in Colombia on 476 adult patients with mild symptoms of COVID-19 showed that treatment with 300 μg/kg per day of Ivermectin for five days did not significantly improve duration of symptoms.[53] However, a meta-analysis of 18 clinical trials evaluating the

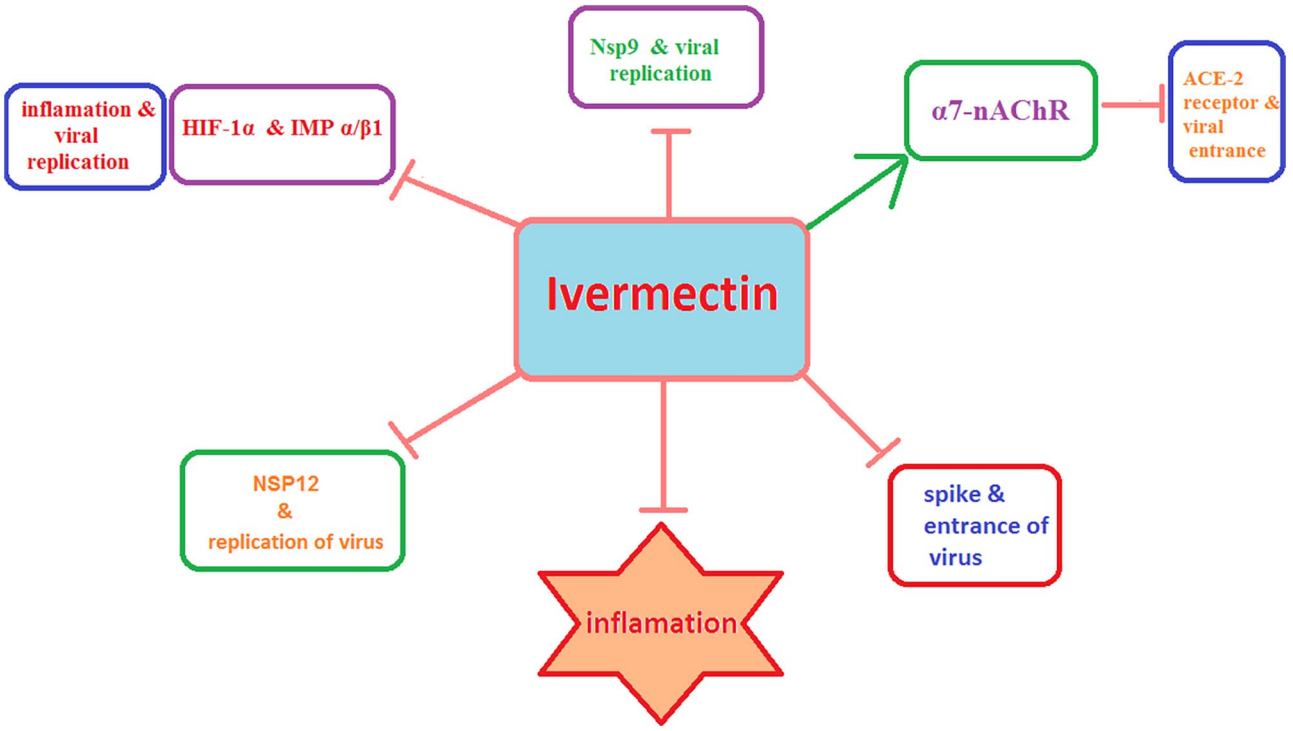

Figure 1. The main molecular targets of Ivermectin. Ivermectin blocks the SARS-CoV-2 proteins involved in the virus replication including RNA-dependent RNA polymerase enzyme NSP12 and Nsp9. It can also block the virus spike protein and whereby inhibits the cell entry of the virus. In addition, Ivermectin enhances the activity of the host A7-nAChR, thereby reducing the ACE2-mediated virus entrance. Moreover, Ivermectin can inhibit the cytoplasmic-nuclear shuttling of the host HIF-1α and viral proteins by disrupting IMPα/β1 complex, thereby reducing viral replication and inflammation. (A color version of this figure is available in the online journal.)

effect of Ivermectin therapy in hospitalized patients with COVID-19 has shown a 68% reduction in mortality correlated with its usage.[54] Of note, many of the physicians announced their personal experience of using Ivermectin. They believed that the results were satisfying for their patients. They proposed that Ivermectin 12 mg twice daily, alone or with other drugs for five to seven days may be safe for mild–moderate or severe cases of COVID-19 infection in all phases of the disease. They also pointed out that the drug is cost-effective and always available. Moreover, it is being used both prophylactically and for treatment in COVID-19 infection.[32,33,35] Notably, Ivermectin is now approved in Peru for at least mild cases of COVID-19. Almost 40 clinical trials are ongoing around the world for evaluating the results of COVID-19 treatment with Ivermectin.[32]

Pharmacokinetics of Ivermectin

Ivermectin is found to have low bioavailability. In the blood circulation, 93% of Ivermectin binds to plasma proteins, so a low amount of Ivermectin remains free to be uptaken by the target cells.[55] The plasma concentration of free Ivermectin would be highly lesser than the concentration required to decrease SARS-CoV-2 replication *in vitro*.[56] After a single oral dose of 150–200 μg/kg of Ivermectin, the plasma concentrations would reach 30–47 ng/mL.[48] Single doses of Ivermectin up to 120 mg are known to be safe and are 10-fold greater than the approved dose by the United States Food and Drug Administration (US FDA), but the C_{max} value would be ~ 250 ng/mL, which is lower than effective *in vitro* concentrations (IC_{50}: 2.5 μM) against SARS-CoV-2.[28,45,46] Notably, after 200 μg/kg injection of Ivermectin in calves, the concentration reached 100 ng/g (about 0.1 μM) in lung tissue, which is not sufficient to get the antiviral effect.[56]

Mechanisms behind ameliorating effect of Ivermectin on COVID-19 infection

Mechanistic studies have shown that Ivermectin can exert therapeutic effects on both the early stages, including SARS-CoV-2 cell entry and replication, and the late cytokine storm-associated phase of COVID-19. Moreover, Ivermectin has the potential to inhibit viral replication and inflammatory responses through blocking the host cell's molecular targets (Figure 1). Mechanisms underlying just-mentioned inhibitory actions of Ivermectin on SARS-CoV-2 infection will be discussed in the following sections.

Ivermectin directly inhibits the early stages of COVID-19 infection

Molecular ducking studies along with experimental investigations have indicated that Ivermectin can interact with viral proteins that play important roles in the SARS-CoV-2 infection, including those involved in viral replication and/or in the entrance of the virus into the host cells. Ivermectin has been predicted to inhibit the viral entry via hindering the interaction between spike protein and host ACE2, thus directing the virus toward a fruitless infection due

to its inability to infiltrate the host cells.[57] While the exact underlying mechanism has yet to be known, docking studies predicted a high binding affinity of Ivermectin for spike protein via hydrogen interaction with Asn487 in the receptor-binding domain (RBD)[58] and with several other residues (LEU492, GLN493, GLY496, TRY505).[59] Moreover, there is an interaction between the alkyl group from Ivermectin and aromatic rings of spike protein residues (TYR449, TYR489, PHE456, LEU455, PHE490). Other docking complex has also predicted five nonpolar interaction residues (Tyr439, Tyr481, Tyr491, Phe483, Leu441) and five polar interaction residues (Arg389, Lys403, Gln479, Gly482, Gln484) had took place between Ivermectin and RBD-spike protein.[58] Among abovementioned residues, the LEU455 and GLN493 possess a high binding affinity with ACE2. In addition, Ivermectin was also found to interact with leucine 91 in the spike protein and histidine 378 of the SARS CoV-2-ACE2 receptor complex.[60] Consequently, Ivermectin may interfere with the attachment of the viral spikes to the human ACE2 receptor, subsequently blocking viral entry, and effectively decreasing the viral infection.[61]

After infection, the SARS-CoV-2 binds to the ACE2 receptor by the virus spike protein, subsequently recruiting the host ribosome and reprogramming it to translate the viral RNA to the functional polypeptides.[62] These polypeptides must be auto-cleaved by 3-chymotrypsin like protease (3CLpro) and papain-like proteases (PLpro) to produce NSP12 needed for the viral replication in infected host cells, such as the RNA-dependent RNA polymerase (RdRp) enzyme NSP12.[62] RdRp, called RNA replicase, is a key enzyme in the virulence of RNA viruses since it mainly acts to catalyze the replication of RNA from a primary RNA template in the virus, thereby blocking viral replication. Nsp12, a conserved protein in coronavirus, is an RdRp and responsible for coronavirus transcription or replication.[63] Importantly, RdRp was already predicted as a significant drug target for inhibiting the replication of MERS-CoV and SARS-CoV.[64] More recently, further studies indicated that Ivermectin has a high binding affinity to the Nsp12-RdRp in SARS-CoV-2, interfering with the virus replication.[65]

In addition, Ivermectin was also predicted to block the active sites of the virus 3CLpro, which disrupts the viral replication, same as antiviral drugs such as boceprevir, ombitasvir, paritaprevir, tipranavir that have inhibitory effect toward 3CLpro enzyme.[66] An *in silico* study indicated that Ivermectin (50 μM) inhibited activity of 3CLpro in SARS-CoV-2 by more than 85%.[66] Docking studies also revealed that Ivermectin via its carbonyl group forms stable hydrogen bonds with the active site residues (Cys145 and His41),[66] polar and nonpolar residues of subunit 1, and subunit 2 residues (Thr304 and glu166) of 3CLpro, destabilizing the complex,[58] resulting in the loss of function and consequently decreasing its protease activity.[67,68] Recently, blocking the 3CLpro complex has been shown a success in the treatment of SARS-CoV and HIV-1, suggesting 3CLpro a viable target for Ivermectin.[69] Of note, 3CLpro structure is highly conserved among coronaviruses, particularly between SARS-CoV-2 and SARS-CoV. Notably, mutation sequences of 3CLpro protein have shown to be fatal for viruses; thus, the risk of drug resistance from virus evolution is markedly decreased.[70]

In addition, the nonstructural protein 9 (Nsp9) is an oligosaccharide/oligonucleotide binding protein that plays an indispensable role in the viral replication. Besides the capacity of Nsp9 to bind viral genomic RNA and mediate viral replication, it can also affect host ribosomal assembly and methylation of mitochondrial rRNA, thereby preventing mitochondrial protein synthesis and thus oxidative phosphorylation, leading to the ATP lowering and the energy loss. Such an effect of Nsp9 has been found to be associated with the symptoms of SARS-CoV-2, including decreased blood pressure following coma, promotion of platelet aggregation, and elevated blood coagulability.[63] These findings together with molecular docking studies showing a strong affinity of Ivermectin to Nsp9 protein suggest this protein as a potential target for the therapeutic armamentarium of the COVID-19 infection.[71]

Ivermectin inhibits the inflammation-associated phase of COVID-19

There is growing evidence indicating the anti-inflammatory effects of Ivermectin against the SARS-CoV-2 infection. Toll-like receptors (TLRs) present intracellularly on the host immune cells bind with the virus and detect the viral attack. TLR4 can be activated by SARS-CoV-2 and also by bacterial lipopolysaccharide (LPS) (detected during ICU settings), leading to activation of interferon (INF) regulatory factors (IRFs), nuclear factor kappa B (NF-κB) pathway, and mitogen-activated protein (MAP) kinases causing elevated gene expression of the IFNs as well as pro-inflammatory cytokines and chemokines responsible for cytokine storm.[72,73] TLR-mediated activation of IRF transcription is a natural antiviral response of a cell. The produced INFs bind to the IFN-I and IFN-III receptors present on neighboring cells, further activating the downstream STAT signaling pathways, defending against the viral attack. In a normal condition and upon recognition of foreign viruses by the host cell TLRs, STAT1, and STAT2 proteins are predominantly activated and form a transcription factor complex (STAT1/STAT2/IRF9) to translocate into the nucleus and upregulate IFN-stimulated genes (ISGs) to interfere with the viral replication.[73,74] Of note, Ivermectin is able to induce the expression of many ISGs, including OASL, IRF9, ISG20, IFIT1, IFIT2, and IF144.[75]

For a virus to cause an infection, such antiviral response should be blocked by inhibiting the IFN production. Upon infection, SARS-CoV-2 proteins antagonize the antiviral IFN signaling,[76] consequently, the cells neighboring the infected cell lose out to receive antiviral IFN signals, permitting SARS-CoV-2 virus replication and spread without any barrier (Figure 2). This is one of the main reasons that, at this stage, COVID-19 infection is "hard to detect" clinically.[77] Indeed, the activity of STAT1 and STAT2-mediated IFN response in the host cell is inhibited by SARS-CoV-2 proteins such as the nonstructural protein 1 (NSP1), ORF3a, and ORF6, causing the hyperactivation of STAT3-mediated responses that result in the cytokine storm and a cascade of deleterious events.[76,78,79] STAT3 leads to a significant production of inflammatory interleukin (IL)-6 cytokine thorough the elevated macrophage activity in COVID-19 patients.[76] STAT3 is also able to activate integrin αvβ6 and thrombospondin at the

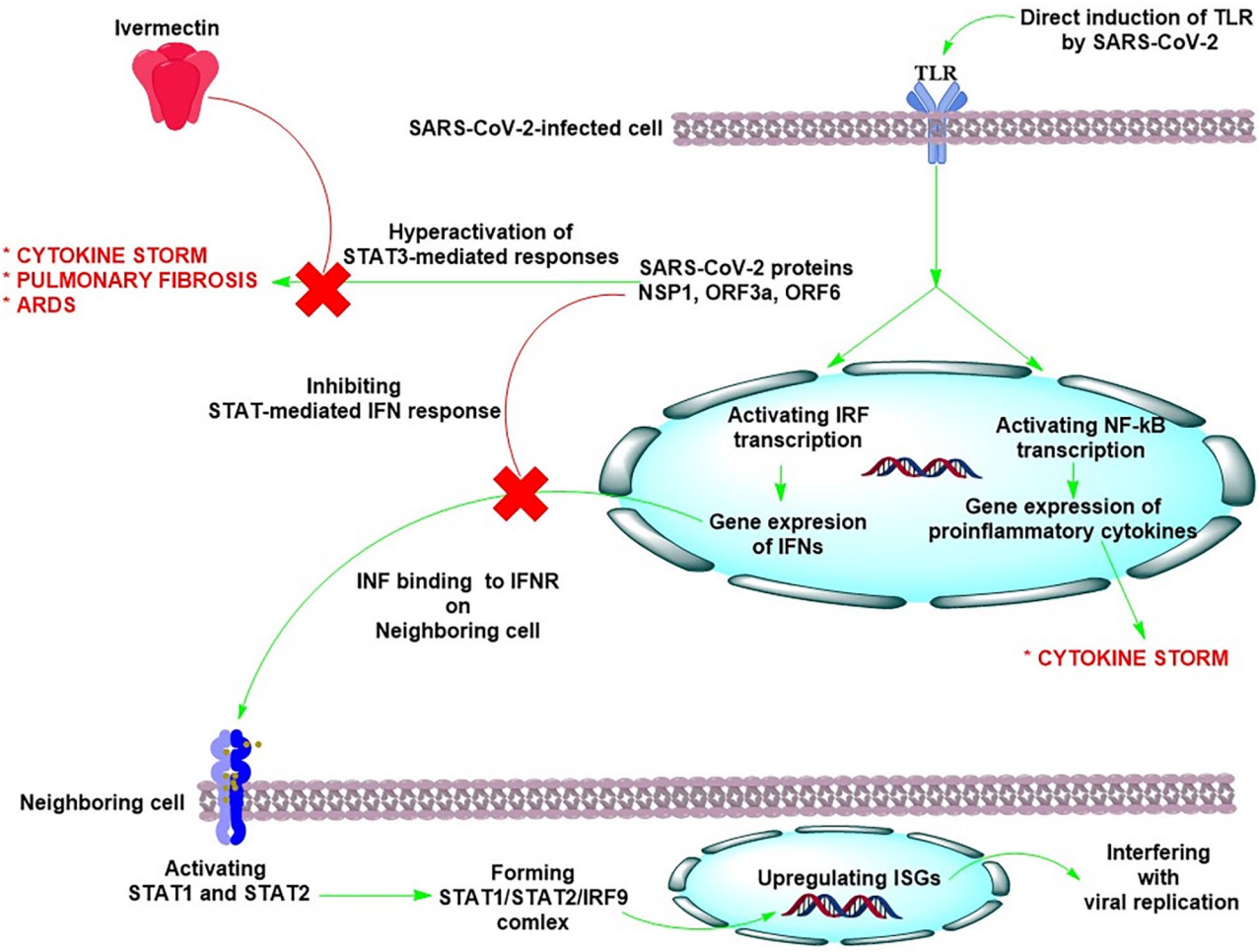

Figure 2. SARS-CoV-2 activates Toll-like receptors (TLR) on immune cells. The TLR activation results in induction of interferon (INF) regulatory factors (IRFs) and nuclear factor kappa B (NF-κB) transcription which upregulate the expression of IFNs and inflammatory cytokines. The produced INFs bind to the IFN-I and IFN-III receptors present on neighboring cells, further activating the downstream STAT1 and STAT2 proteins that form a transcription factor complex (STAT1/STAT2/IRF9) to translocate into the nucleus and upregulate IFN-stimulated genes (ISGs) to interfere with the viral replication. The activity of STAT1 and STAT2-mediated IFN response is inhibited by SARS-CoV-2 proteins such as the nonstructural protein 1 (NSP1), ORF3a, and ORF6, causing the hyperactivation of STAT3-mediated responses that result in the cytokine storm, pulmonary fibrosis, and acute respiratory distress syndrome (ARDS). Ivermectin can block STAT3-mediated responses. (A color version of this figure is available in the online journal.)

lung extracellular matrix, contributing to TGF-β activation and pulmonary fibrosis.[80] The TGF-β activation upregulates the plasminogen activator inhibitor-1 (PAI-1), promoting the continuous expression of IL-6.[80] Eventually, STAT3 and PAI-1 enhance the generation of hyaluronan (HA), causing severe acute respiratory distress syndrome (ARDS) in COVID-19 patients, resulted from diffuse alveolar damage (DAD).[81] Notably, Ivermectin was found to suppress the STAT3 activity, leading to a significant reduction in IL-6 production. In addition, Ivermectin can also induce the ubiquitination-mediated degradation of p21 activated kinase 1 (PAK1), an important mediator that binds to STAT3 for IL-6 gene transcription, interrupting IL-6 expression.[76,82] The decrease of STAT3 levels by Ivermectin could also reduce the level of HA, inhibiting ADRS in COVID-19 patients.[81] Of note, an *in vivo* study showed that Ivermectin can prevent the clinical deterioration through limiting the inflammation of the upper and lower respiratory tracts in SARS-CoV-2-infected hamsters. Transcriptomic analyses of infected lungs revealed that Ivermectin significantly reduced type I and type III IFN responses and modulated other important inflammatory pathways, with a marked reduction of the IL-6/IL-10 ratio and promoting M2 polarization of myeloid cells recruited to the lung.[83]

Besides, in ICU settings with increased possibility of bacterial infections, LPS-mediated inflammation can also worsen SARS-CoV-2-mediated inflammation. Notably, oral Ivermectin was found to dose dependently decrease inflammation and improve survival in mice challenged with a lethal dose of LPS.[84] Such an *in vivo* effect was confirmed by the *in vitro* studies that indicated Ivermectin could inhibit production of inflammatory cytokines by LPS-challenged macrophages.[84–86] *In vivo* and *in vitro* studies showed the ability of Ivermectin to prevent LPS-induced production of inflammatory cytokines including tumor necrosis factor-alpha (TNF-α), IL-6, and IL-1 via suppressing NF-κB and MAP kinase pathways.[84,85,87] These findings not only support anti-inflammatory effect of Ivermectin in the late stages of SARS-CoV-2 infection, but also indicate the protective effect of this drug against ICU-caused bacterial infection in COVID-19 patients. In mechanism, there is evidence that suggests glycine-gated strychnine-inhibitable chloride channels

as a possible target mediating the anti-inflammatory effect of Ivermectin.[54] These glycine receptors are expressed by several kinds of immune cells such as neutrophils and alveolar macrophages as well as vascular endothelium. Activation of glycine receptors on immune and endothelial cells can inhibit inflammatory responses, likely through hyperpolarization of plasma and inhibition of endosomal nicotinamide adenine dinucleotide phosphate oxidase activity.[88,89] Of note, there are findings that show Ivermectin as an agonist for glycine receptors can highly increase the activation of such receptors[90–93] and whereby prevents LPS-mediated inflammation in macrophages.[86]

Ivermectin inhibits molecular targets in lung cells involved in COVID-19 infection

The inhibitory effect of Ivermectin on the host importin (IMP) α/β1 complex has been suggested to be one of the possible mechanisms underlying the antiviral effect of Ivermectin.[41] There are reports that show Ivermectin has the high binding affinity to IMPα and can inhibit activity of IMP α/β1.[58,65] IMP α/β1 transports the host proteins in-and-out of the nucleus. The import of a protein into the nucleus requires nuclear localization signal (NLS) to be recognized by the IMPα from the importin heterodimer complex (IMPα/β1), IMPβ1 alone, or the homologues thereof. From there, the cargo protein interacts with the IMPβ1 and is translocated via the nuclear pore complex (NPC) embedded in the nuclear envelope.[94] This nuclear transport complex has been documented to be hijacked by several viruses, such as HIV-1, Dengue Virus, Influenza, as well as SARS-CoV and SARS-CoV-2 to gain access into the nucleus and to facilitate infection.[95]

Ivermectin has been found to inhibit the cytoplasmic-nuclear shuttling of viral proteins by disrupting IMPα/β1 complex, thereby reducing viral replication.[33,42,44] It has been already found that Ivermectin through this mechanism can affect pseudorabies viruses[40] and a remarkable number of RNA viruses[42,96] such as Dengue Virus 1-4,[97] West Nile Virus,[41] Venezuelan Equine Encephalitis Virus,[37] and Influenza.[38] Of note, this mechanism was also recently found to be responsible for reducing the entrance of SARS-CoV-2 proteins into the nucleus and disturbing the reproduction and survival of SARS-CoV-2 virus.[32,42] Mechanistically, the Ivermectin binds to the IMPα, blocking the formation of the IMPα/β1 complex, thereby inhibiting the IMPα/β1-dependent nuclear transport activities of viral SARS-CoV-2 proteins.[95]

Furthermore, there is also evidence that shows Ivermectin may exert the antiviral activity through inhibiting the formation of the host IMP-α/hypoxia-inducible factor 1-alpha (HIF-1α) complex.[34] HIF-1α is a subunit of a heterodimeric transcription factor that responds to the reduction of oxygen levels in the cellular environment or hypoxia, and adapts the cellular response to oxygen availability.[98–100] Through the virus-mediated activation of HIF-1α, the inflammatory response is exacerbated in severe cases of COVID-19 disease. The targets of HIF-1α are all involved in the activation of pro-inflammatory cytokine expression and the subsequent inflammation.[101] HIF-1α activation leads to metabolic reprogramming and virus replication, and inhibition of HIF-1α reduces viral replication significantly.[102] Notably, formation of the IMP-α/HIF-1α complex is required for efficient nuclear translocation of HIF-1α. Ivermectin has been reported to reduce binding activity of the host HIF-1α to the IMP α/β-heterodimer.[103] Upon Ivermectin treatment, nuclear localization and nuclear levels of HIF-1α, as well as HIF-transcriptional activity and HIF-target gene expression were significantly decreased.[103] Based on such evidence, it was suggested that Ivermectin has the potential to inhibit the SARS-CoV-2 replication and the cytokine-mediated pro-inflammatory response through blocking the formation of HIF-1α and IMP α/β1 complex.[34]

Another host target involving the antiviral mechanism of Ivermectin against SARS-CoV-2 is α7-nAChR. The activation of nicotinic acetylcholine receptors (nAChR), which respond to the neurotransmitter acetylcholine, the α7 subtype, could increase the viral receptor ACE-2 in airway epithelial cells. Indeed, activation of the α7-nAChR subtype induces overexpression of ACE2 and this is the reason for the vulnerability of patients with chronic obstructive pulmonary disease to severe COVID-19; however, the exact mechanism remains still unclear.[104–107] Of note, a low IC_{50} of Ivermectin (0.156 μM) has been found to inhibit the α7-nAChR,[41,108,109] thereby blocking SARS-CoV-2 entrance into the host cells.

Conclusions

Ivermectin could inhibit the proliferation of SARS-CoV-2 *in vitro*, but the IC_{50} is 35-fold higher than the concentration of approved clinical doses (9 mg). After single dose of Ivermectin 120 mg, the C_{max} would be ~250 ng/mL, which is lower than the effective concentrations against SARS-CoV-2 *in vitro*. Although even 2000 μg/kg of Ivermectin (10 times more than FDA-approved dose) was used and rarely CNS toxicity was seen, we should be worried about the probably adverse effects of this drug at higher doses that would be necessary for treating patients with COVID-19.

Although the *in vitro* studies indicate only the effectiveness of high doses of Ivermectin, several clinical studies have declared cost-effective, easy to availability, and promising results of Ivermectin, even with usual doses. Moreover, the reports show mortality reduction, especially in cases that need oxygen or mechanical ventilation. The effectiveness of Ivermectin, with both preventive and therapeutic approaches, in all phases of the disease has also been demonstrated. To decrease the dose and adverse effects, a combination of Ivermectin with other drugs like hydroxyl chloroquine may be effective. In addition, providing an inhaled formulation to deliver a high dose of Ivermectin locally in the lung, could reduce systemic exposure and may get better results and prevent the adverse effects of the drug.

Besides, Ivermectin shows attractive features that can distinguish its therapeutic potential from other antiviral agents against SARS-Cov-2 infection. Of note, a few FDA-approved drugs are being used on COVID-19 patients at the early stage of infection which may cause less severe infection due to reduced entry of COVID-19 into the cell. Moreover, drugs blocking viral replication would be expected to be of lesser utility in the context of the late cytokine storm-associated phase of COVID-19. Interestingly, Ivermectin is useful in

both the early stages and the later stages of the disease. In the early stages, Ivermectin can inhibit SARS-CoV-2 cell entry through blocking both the host molecular targets, such as the α7-nAChR and the ACE2 receptors, and viral molecular targets, such as the spike protein. Ivermectin also can suppress the SARS-CoV-2 replication as evidenced by the reduced viral load in the SARS-CoV-2-infected cells treated by Ivermectin. Molecular and ducking studies indicated that Ivermectin can inhibit the SARS-CoV-2 replication through suppressing the nuclear transfer of SARS-CoV-2 proteins by the host IMP α/β and blocking the activity of the virus proteins including Nsp12-RdRp, Nsp9, and 3CLpro. Ivermectin has also potential to ameliorate the late stage cytokine storm in the COVID-19 patients. Ivermectin has been found to exert anti-inflammatory effect through modulating the signaling pathways and mediators which involve in the inflammatory responses induced by SARS-CoV-2, particularly STAT signaling and chloride channel.

AUTHORS' CONTRIBUTIONS

AAM-B contributed to the conception and design of the work. NB and MM performed the database search and preparing the manuscript. NB and SC revised the manuscript and prepared the figures. All authors read and approved the final manuscript.

DECLARATION OF CONFLICTING INTERESTS

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