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REVIEW

A cellular and molecular biology-based update for ivermectin against COVID-19: is it effective or non-effective?

Mohsen Nabi-Afjadi[1] · Fatemeh Mohebi[2] · Hamidreza Zalpoor[3,4] · Fatemeh Aziziyan[1,4] · Abdullatif Akbari[4] · Hemen Moradi-Sardareh[5] · Elham Bahreini[6] · Amir Mansour Moeini[7] · Hossein Effatpanah[5]

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Abstract

Despite community vaccination against coronavirus disease 2019 (COVID-19) and reduced mortality, there are still challenges in treatment options for the disease. Due to the continuous mutation of SARS-CoV-2 virus and the emergence of new strains, diversity in the use of existing antiviral drugs to combat the epidemic has become a crucial therapeutic chance. As a broad-spectrum antiparasitic and antiviral drug, ivermectin has traditionally been used to treat many types of disease, including DNA and RNA viral infections. Even so, based on currently available data, it is still controversial that ivermectin can be used as one of the effective antiviral agents to treat severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or not. The aim of this study was to provide comprehensive information on ivermectin, including its safety and efficacy, as well as its adverse effects in the treatment of COVID-19.

Keywords COVID-19 · Ivermectin · SARS-CoV-2 · Favipiravir · Ribavirin · Famotidine

Abbreviations

SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
COVID-19	Coronavirus disease 2019
RdRp	RNA-dependent RNA polymerase
NSP	Nonstructural protein
IMPα	Importin alpha
NF-κB	Nuclear factor kappa B
VEGF	Vascular endothelial growth factor
ETC	Electron transport chain
OXPHOS	Oxidative phosphorylation
MERS-CoV	Middle East Respiratory Syndrome

Mohsen Nabi Afjadi and Fatemeh Mohebi have contributed equally to this work.

✉ Mohsen Nabi-Afjadi
Mohsennabi66@gmail.com

✉ Hemen Moradi-Sardareh
hemen.moradi@yahoo.com

✉ Elham Bahreini
elhambahreini@yahoo.com

Hamidreza Zalpoor
hamidreza.zlpr1998@gmail.com

Amir Mansour Moeini
am.moeini.md@gmail.com

[1] Department of Biochemistry, Faculty of Biological Sciences, University of Tarbiat Modares, Tehran, Iran

[2] Molecular Medicine Research Center, Hormozghan Health Institute, Hormozghan University of Medical Sciences, Bandar Abbas, Iran

[3] Shiraz Neuroscience Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

[4] Network of Immunity in Infection, Malignancy and Autoimmunity (NIIMA), Universal Scientific Education and Research Network (USERN), Tehran, Iran

[5] Asadabad School of Medical Science, Hamedan, Asadabad, Iran

[6] Department of Biochemistry, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran

[7] Department of Internal Medicine, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran

Introduction

Coronavirus Infectious Disease 2019 (COVID-19) is a fatal respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Esakandari et al. 2020; Khezri et al. 2022; Khomari et al. 2021; Zhou et al. 2020). COVID-19 began in December 2019 when an unknown cause of pneumonia broke out in Wuhan City, Hubei Province, China. All early reports of the source of the disease came from the Huanan Seafood Wholesale Market, which sells aquatic products, live birds, and wild animals. Fever, headache, cough, and shortness of breath are common symptoms of COVID-19 that can accompany multiple organ failure (Hui et al. 2020). In addition, recent studies have suggested that COVID-19 may lead to the progression of multiple cancers and other diseases (Zalpoor et al. 2022a, 2022b, 2022c, 2022d, 2022e, 2022f, g).

Proteomic and genetic analysis of SARS-CoV-2 showed 94.6% and 79.5% similarity in nonstructural protein amino acid sequence and nucleic acid structure, respectively, between SARS-CoV-2 and other SARS species. SARS-CoV-2 is a positive-stranded RNA virus (+ssRNA virus) with sequences that translate directly into viral proteins. The five gene segments of the RNA genome are cloning enzymes and four structural proteins, including spike protein (S), membrane protein (M), envelope protein (E), and nucleocapsid protein (N). The replicase gene (ORF1ab gene) encodes 16 nonstructural proteins (NSP 116) that are over 21 kb in size and are translated into pp1ab polyproteins. Replicase (pp1a), an RNA-dependent RNA polymerase (RdRp), is used to synthesize a negative-sense antigenome during genome replication, which serves as a template for generating a novel positive-sense viral genome. The NSP1 protein binds to the 40S subunit of the ribosome in the cell and inhibits translation in the host cell. By inhibiting gene expression in host cells, the NSP1 protein promotes viral gene expression in infected cells and evades the host's immune response. The NSP2 protein is involved in the regulation of cell survival transduction pathways through interactions between host PHB and PHB2 molecules. These two proteins play important roles in stabilizing mitochondrial function and protecting cells from stress. PLPRO, located next to NSP3, has either deubiquitinating or deISGilating activity, which is involved in suppressing the immune response. The NSP2 protein is involved in the regulation of cell survival transduction pathways through interactions between host PHB and PHB2 molecules. These two proteins play important roles in stabilizing mitochondrial function and protecting cells from stress. PLPRO, located next to NSP3, has either deubiquitinating or deISGilating activity, which is involved in suppressing the immune response. This protein, along with the NSP4 protein, is also involved in the formation of membrane vesicles required for viral replication. NSP3 protein suppresses induction of type 1 interferon and innate immunity by blocking phosphorylation, dimerization, and nuclear–cytoplasmic transition. This protein is involved in inhibiting nuclear factor kappa B (NF-κB) message transmission. The spike glycoprotein has two subunits: the S1 protein for binding to the receptor and the S2 protein for the fusion of the viral envelope with the host cell membrane. Viral infection begins with the binding of the S1 protein to the hACE2 receptor (angiotensin converting enzyme 2) and a conformational change of the S glycoprotein, followed by proteolysis of the S glycoprotein by CatB/L (cathepsins B and L) and TMPRRS2 with the S2 fusion peptide and activate the virus. Membrane is integrated into the endosome (Mielech et al. 2014; Payandeh et al. 2021; Wang et al. 2020; Wu et al. 2020).

Ivermectin is a broad-spectrum anthelmintic, antibacterial, and antiviral agent approved by the FD (Campbell and Benz 1984; Crump and Omura 2011). This drug has been shown to be effective against several DNA and RNA viruses, including SARS-CoV-2, in vitro. Ivermectin acts on SARS-CoV-2 by preventing the pathogenic protein/viral genome from entering the nucleus of the host cell (Caly et al. 2020; Lv et al. 2018; Raza et al. 2020; X. Wang et al. 2019).

The current review summarizes the past and present use, mechanisms, and progress of preclinical studies and clinical trials of ivermectin for the treatment of COVID-19. Although there are insufficient data available to explain the in vivo activity of ivermectin on SARS-CoV-2. Results demonstrating the antiviral activity of ivermectin in vitro against SARS-CoV-2 and in vivo effects against other similar viruses provide investigators with sufficient confidence that laboratory and clinical studies of the use of ivermectin for the treatment of COVID-19 are still ongoing.

Ivermectin prevents SARS-CoV-2 entry into the host cells

Before SARS-CoV-2 attaches to the host cell, two molecules of ivermectin interact with each other in a “tail” mode and form an ionospheric activated chloride channel, triggering apoptosis and osmotic cell death (Dominguez-Gomez et al. 2018; Dueñas-González and Juárez-Rodríguez 2021; Rizzo 2020). Additionally, in silico studies of the effects of ivermectin on SARS-CoV-2 have shown that several strategies prevent the virus from entering the host cell. First, ivermectin binds to the leucine 91 region of the S glycoprotein and the histidine 378 region of the host cell ACE2 receptor (the specific receptor of SARS-CoV-2) (Nabi-Afjadi et al. 2022). Moreover, ivermectin showed the highest affinity for S glycoprotein, RdRp, NSP14, and TMPRSS2 active sites with higher H-bond formation compared to ivermectin,

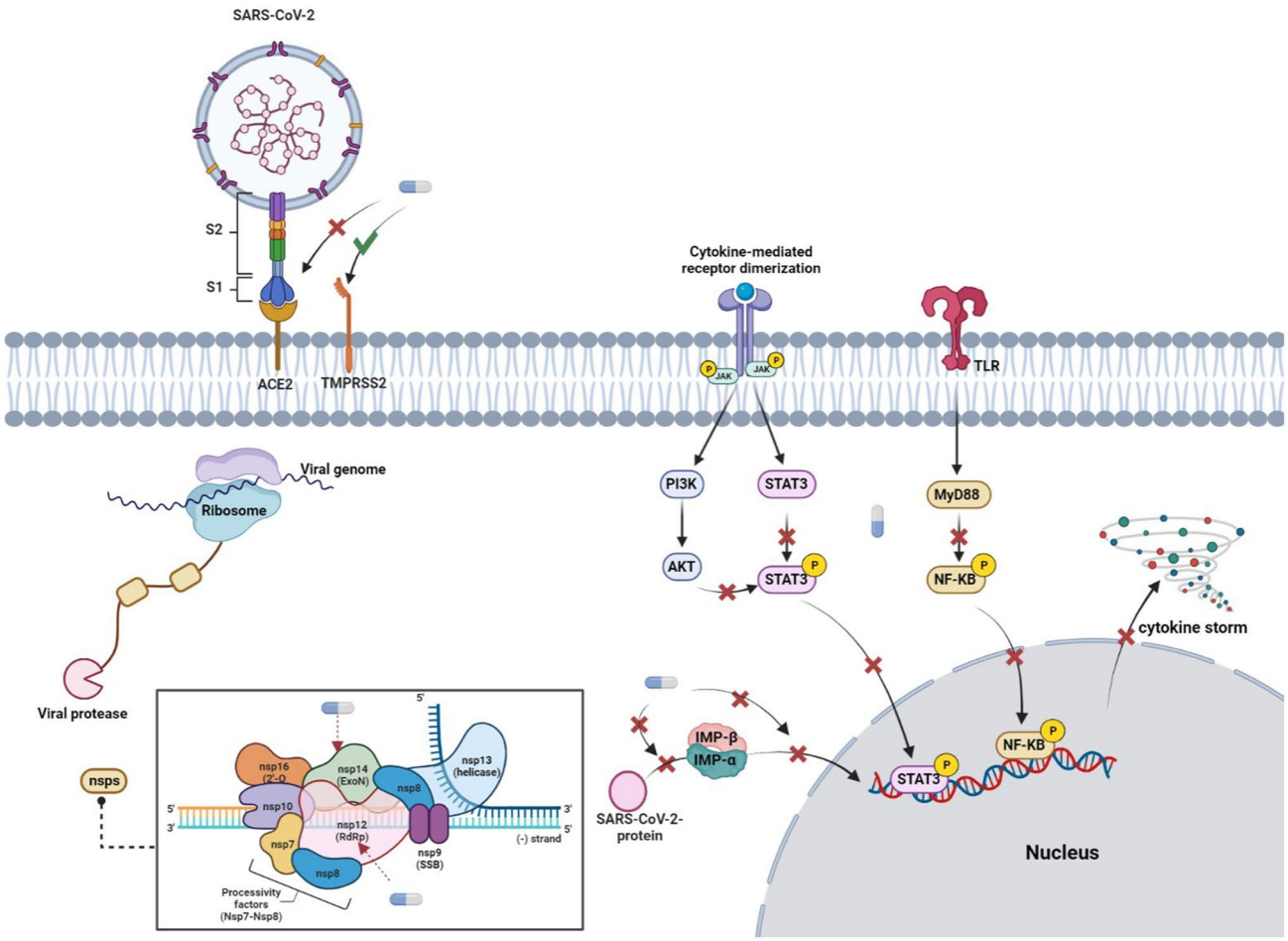

Fig. 1 Ivermectin effects on SARS-CoV-2 entry to host cells, reproduction, and anti-inflammatory response with targeting JAK/STAT, PI3K/AKT, and NF-κB signaling pathways and transcriptional activity of STAT and NF-κB

chloroquine, favipiravir, remdesivir, and hydroxychloroquine (Choudhury et al. 2021; Eweas et al. 2021; Lehrer and Rheinstein 2020; Zaidi and Dehgani-Mobaraki 2021). By interfering with its ability to bind its relevant ligands, Choudhury et al. demonstrated that ivermectin is also effective against human proteases, replicases, and TMPRSS2 receptors (Choudhury et al. 2021).

Importin alpha (IMP α) is another molecular target of ivermectin for SARS-CoV-2. Ivermectin has been shown to specifically prevent IMP α/β-mediated nuclear transport in HIV-1 replication and Dengue infection. Therefore, by the same mechanism in the mentioned virus, ivermectin was expected to inhibit SARS-CoV-2 (King et al. 2020; Wagstaff et al. 2012). A study by Young et al. confirmed the effect of ivermectin on IMPα in host cells. They showed that ivermectin inhibited the correlation between IMPα and IMPβ, but was also able to dissociate IMPα/β heterodimers. The following study using CD spectroscopy revealed that the armadillo (ARM)-rich domain of IMPα was the specific binding site of ivermectin. In addition, as the concentration of ivermectin increased, the breakage of the alpha helix in ARM significantly increased, but there was no change in the structure of IMPβ. In addition, the effect of ivermectin on IMPα was demonstrated to prevent binding to NLS, including the dengue virus NSP5. Therefore, it can be said that the effect of ivermectin on SARS-CoV-2 is similar to the prevention of N interaction with IMPα (S. N. Yang et al. 2020a, b) (Fig. 1).

Ivermectin inhibits SARS-CoV-2 reproduction

Other viruses that were able to enter the host cell may be affected by other ivermectin schemes. As mentioned above, ivermectin interacts with RdRP (binding energy − 9.7 kcal/mol) located on NSP12 to serve as an important enzyme in the replication and transcription of SARS-CoV-2. Ivermectin also interacts with NSP14 to act as a capping of viral RNA through corrective exoribonuclease and methyltransferase

activity. Thus, ivermectin inhibits SARS-CoV-2 replication by interfering with an important protein/replication factor of the virus. In host cells, SARS-CoV-2 RNA is translated into a polyprotein in which specific enzymes are autoproteolytically cleaved, facilitating the separation of the enzyme responsible for viral replication from the polyprotein. Chymotrypsin-like protease (3'cl pro/Mpro) is one of the enzymes that prevents the binding of ivermectin. It also efficiently binds to two proteins, Mpro and, to a lesser extent, SARS-CoV-2 PLpro. Thus, it serves to prevent post-translational processing of viral polyproteins. In addition, ivermectin binds to Mpro and PLpro of the SARS-CoV-2 polyprotein as an important protease in post-translational processing (Eweas et al. 2021; Ma et al. 2015; Mody et al. 2021; Swargiary 2020; V'kovski et al. 2021). It has been suggested that autophagy may promote the invasion and proliferation of SARS-CoV-2 into host cells, which may be exacerbated by ivermectin (Yang and Shen 2020). In addition, studies have shown that ivermectin may induce autophagy via the AKT/mTOR signaling pathway (Dou et al. 2016; Liu et al. 2019). Therefore, these findings suggest that ivermectin may play a double-edged role, and further studies are needed to confirm the positive or negative effects of ivermectin on SARS-CoV-2 invasion and replication in host cells.

Ivermectin role in immune system response and anti-inflammatory effects

Ivermectin acts as an anti-inflammatory agent by inhibiting NF-κB, AKT/mTOR, STAT3, and interferon-dependent pathways, reducing the production of proteins associated with inflammation, significantly reducing the severity and mortality of COVID-19. For example, TNF-α, IL-1ss, IL-4, IL-5, IL-6, and IL-13. Studies have shown that ivermectin regulates the cell-mediated and humoral immune responses of a variety of bacterial, viral, parasitic, and neoplastic diseases (Sajid et al. 2006; Stankiewicz et al. 1995). Ivermectin has been shown in vivo and in vitro to have anti-inflammatory properties by inhibiting the increase in NF-κB activity and inhibiting the production of TNF-α, IL-1 and IL-6 (Zhang et al. 2008). NF-κB is activated by SARS-CoV-2 through pattern recognition receptors (Nabi-Afjadi et al. 2021). When SARS-CoV-2 binds to ACE2, ACE2 decreases on the cell surface, resulting in increased AngII expression. In addition to NF-κB activation by SARS-CoV-2 infection, the type 1 angiotensin receptor axis may play a role in inducing the production of TNF-α and soluble IL-6Ra (sIL-6Ra) via disintegrin and metalloprotease 17 (ADAM17) (Eguchi et al. 2018). In non-immune cells, IL-6 binds to sIL-6R and activates signal transducer and transcriptional activator 3 (STAT3) (Joshi et al. 2021a, b). STAT3 and NF-κB can activate IL-6 enhancers to produce other proinflammatory cytokines and chemokines, including monocyte chemoattractant protein (MCP1), IL-8, and vascular endothelial growth factor (VEGF) (Murakami et al. 2019). When PAK1 binds to both JAK1 and STAT3, the PAK1/STAT3 complex is formed, which activates transcription of the IL-6 gene required for cytokine storm during COVID-19 infection (Kim et al. 2019). Ivermectin has been shown to attenuate the cytokine storm in COVID-19 by inhibiting Akt/mTOR signaling and promoting ubiquitin-mediated PAK1 degradation, thereby interfering with STAT3 activity and IL-6 production (Dou et al. 2016).

When SARS-CoV2 enters the host cell, it intercepts the function of the host cell and inhibits the IFN-mediated antiviral response of the host cell. SARS-CoV-2 proteins, including ORF3a, ORF6, and NSP1, block IFNI signaling (Konno et al. 2020; Yang et al. 2020a, b). Thus, cells surrounding infected cells cannot receive the IFN protective signal, allowing SARS-CoV-2 to freely spread and proliferate. Many IFN-related genes have been shown to be induced by ivermectin, including IFIT1, IFIT2, IF144, ISG20, OASL, and IRF9 (Seth et al. 2016). Collectively, based on the available evidence, in addition to its antiviral effect, ivermectin has the potential to improve protective IFN signaling and has anti-inflammatory properties, which could be effective in reducing the side effects of the cytokine storm and inflammatory response associated with coronavirus. Nevertheless, ivermectin can induce activation of P2X7 receptor and downstream signaling transduction, resulting in inflammatory and pathological effects. Ivermectin has been shown to increase ATP sensitivity and delay the inactivation of current after ATP dissociation at the PX24 receptor. This occurs at the same time as the enhancement of ATP-induced current and Ca2 + signaling at the P2X7 receptor (Juarez et al. 2018). In addition, Zalpoor et al. (Zalpoor et al. 2022c) reported that overactivation of P2X7 receptors may be involved in the pathological and inflammatory responses in SARS-CoV-2 infection. Therefore, more studies are needed to approve the inflammatory or anti-inflammatory effects of ivermectin in patients with COVID-19.

Ivermectin effects in energy metabolism pathways associated with COVID-19

Recent analysis has shown the activation of glycolysis, the TCA cycle, and oxidative phosphorylation chain during COVID-19 (Gardinassi et al. 2020). Codo et al. demonstrated that the expression of genes involved in glycolysis is upregulated in cultures of SARS-CoV-2-infected bronchoalveolar lavage monocytes from COVID-19 patients (Codo et al. 2020). In addition, another similar study showed that SARS-CoV-2 infection stimulated glycolysis specifically in monocytes. For some COVID-19 infections, changes in their pathway may contribute to the pathology

and severity of the disease. The Krebs cycle may be considered as the immune-metabolic center of macrophages. The intermediate products of the Krebs cycle, succinate and citric acid, accumulate in proinflammatory macrophages. The non-metabolic signaling roles of these metabolites play an important role in the expression of inflammatory genes (Ryan and O'Neill 2020). Therefore, targeting these metabolic processes could help develop potential treatments to alleviate the inflammatory response and side effects during COVID-19. Ivermectin has recently been shown to play an important role throughout molecular networks by acting on key enzymes in energy metabolism pathways, particularly glycolysis phosphofructokinase, platelet (PFKP), isocitrate dehydrogenase [NADP (+)] 2 (IDH2) and isocitrate dehydrogenase, [NAD(+)] 3-non-catalytic subunit beta (IDH3B), cytochrome b (CYTB), NADH dehydrogenase 2 (ND2), (ND5) and ubiquinol cytochrome c reductase hinge protein (UQCRH) oxidation of the Krebs cycle Phosphorylation, and monocarboxylate transporter 1 (MCT1) and MCT4 in the lactate shuttle(Zhan and Li 2021). Therefore, we hypothesized that ivermectin might have a therapeutic effect on COVID-19 by altering energy metabolic pathways, reducing the inflammatory response, and reducing some of the side effects of COVID-19 (Fig. 2).

Ivermectin also plays a role in causing oxidative stress and apoptosis, according to several studies (Atakisi et al. 2009; El-Far 2013). Ivermectin, for example, suppresses

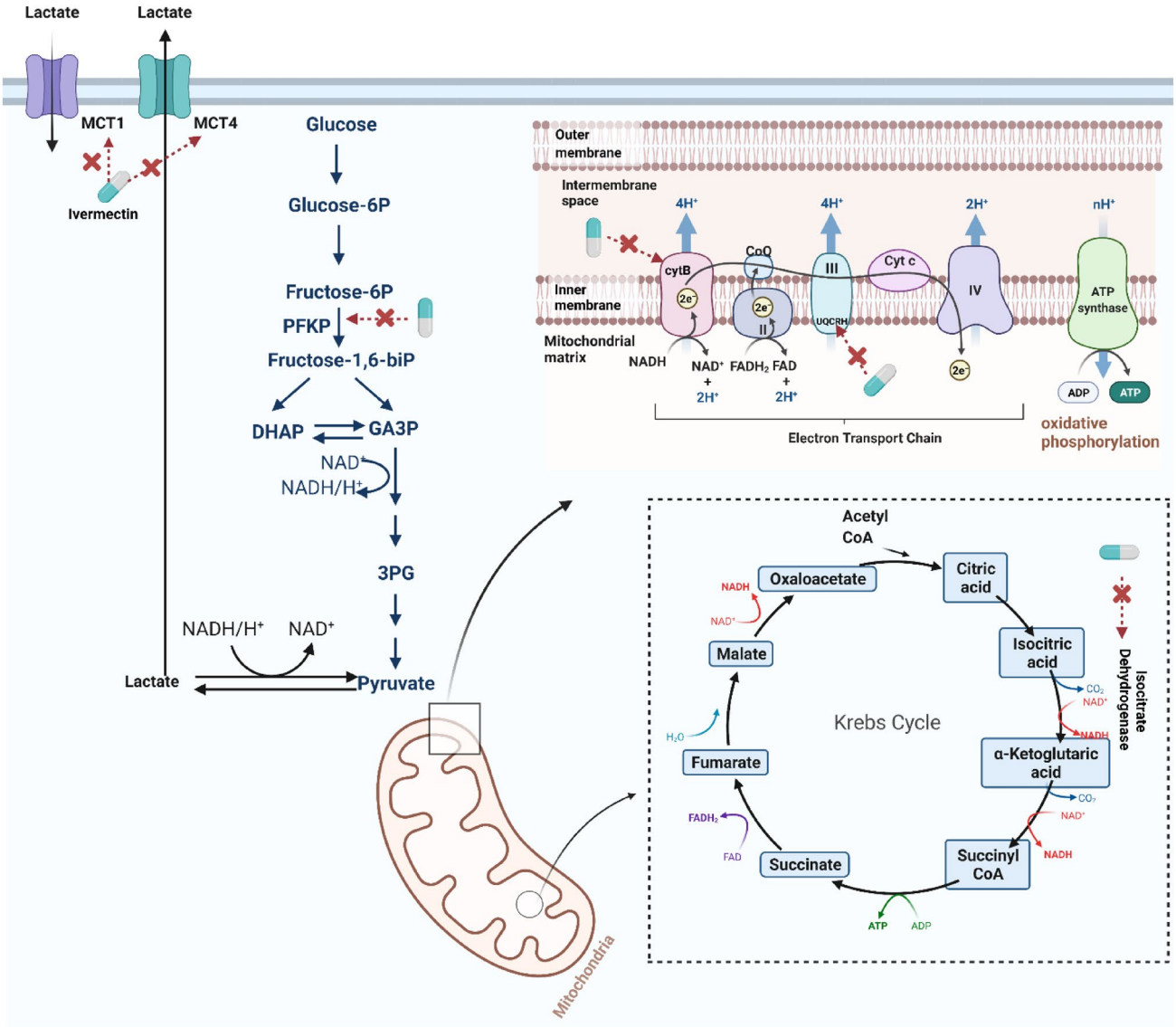

Fig. 2 Ivermectin effects on energy metabolism pathways associated with COVID-19; Krebs cycle, glycolysis, electron transport chain (ETC), and oxidative phosphorylation (OXPHOS)

cell proliferation in colorectal cancer cells by promoting ROS-mediated mitochondrial apoptosis and causing S-phase arrest, as reported in Shican Zhou et al. (Zhou et al. 2021). Further, antibiotics and anthelmintic drugs which prevent DNA or RNA replication in bacteria act on human cancer cells by targeting mitochondria. In glioblastoma cells and brain endothelial cells, ivermectin increases mitochondrial superoxide levels due to mitochondrial dysfunction. In glioblastoma and endothelial cells, the presence of antioxidants reversed the inhibitory effect of ivermectin, showing that the presence of antioxidants is required to reverse its action (Liu et al. 2016). During the early stages of pregnancy, ivermectin may also interfere with normal placentation processes due to constant exposure and accumulated levels. The authors demonstrated that ivermectin adversely affects pTr and pLE cells, including cell cycle arrest, apoptosis, mitochondrial permeabilization, calcium accumulation, endothelial dysfunction, and disruptions in cellular homeostasis caused by mitochondrial dysfunction. In this way, ivermectin should be investigated more for pregnant women who have been infected with SARS-CoV-2 (Lee et al. 2019).

Clinical trials of ivermectin

Due to insufficient human clinical evidence for ivermectin, more clinical trials are required to determine its efficacy. While some researchers have shown ivermectin to be helpful in treating COVID-19, others have reported it to be ineffective. For example, Rajter et al. studied 280 patients with COVID-19 at four Broward Health Florida hospitals from March 15 to May 11, 2020. In this study, patients were divided into two groups. 173 patients received ivermectin and 107 received controls. Hydroxychloroquine, azithromycin, or both drugs were administered together to control and intervention patients. The results showed that ivermectin could significantly reduce mortality in both general and severe lung disease patients compared to controls. There was no significant difference in length of hospitalization between the two groups (Rajter et al. 2021). However, this outcome for hospitalization differs from a randomized double-blind trial by Shahbaznejad et al. A single dose of ivermectin was studied in Mazandaran, Iran, in 69 patients with COVID-19. This study showed that mean dyspnea, persistent cough, hospitalization, and incidence of lymphopenia were significantly reduced in patients treated with ivermectin (Shahbaznejad et al. 2021b). To investigate the rate and safety of viral load reduction of ivermectin (Ahmed et al.). A randomized, double-blind, placebo-controlled trial was conducted in 72 hospitalized patients in Dhaka, Bangladesh. In this study, patients were divided into three groups. The first group received 12 mg ivermectin once daily for 5 days, the second group received a single dose of ivermectin (12 mg) plus 200 mg doxycycline on the first day, and the next day 100 mg every 12 h only doxycycline was administered. As a result, the safety and effectiveness of ivermectin were confirmed as well as the effect of ivermectin on the virus clearance rate and improvement of clinical symptoms in the treatment of mild COVID-19 in adult patients (Ahmed et al. 2021). Okumus et al. aimed to find genetic mutations that affect ivermectin metabolism and its toxic effects in patients with severe COVID-19 pneumonia, as well as to evaluate the efficacy and safety of ivermectin in unmutated patients. Their study was a prospective, randomized, and single-blind trial, included both study and control groups. They reported that ivermectin could improve clinical recovery, improve prognostic test indicators, and reduce mortality in patients with severe COVID-19. They suggested that ivermectin should be explored as an alternative drug to treat COVID-19 disease or as a complement to an existing protocol (Okumuş et al. 2021).

Despite these results, Mohan et al. refute the beneficial effect of ivermectin in reducing viral load, through a search of randomized, double-blind controlled trials in 125 hospitalized patients with mild-to-moderate COVID-19. In this study, patients were randomly divided into two groups: recipients of ivermectin (24 or 12 mg daily) and placebo. After 5 days, the reverse transcriptase-polymerase chain reaction (RT-PCR) test was negative for 47.5% in the ivermectin 24 mg group, 35.0% in the ivermectin 12 mg group, and 31.1% in the placebo group. The difference between groups was not statistically significant, p value = 0.30. Compared with placebo, oral doses of ivermectin increased negative RT-PCR tests or decreased viral loads in patients receiving COVID-19, but these differences between groups were not statistically significant (18). Another double-blind, randomized, placebo-controlled trial in Indian patients with mild-to-moderate disease COVID-19 was conducted by Ravikirti et al. Patients in the intervention group received 12 mg of ivermectin on days 1 and 2, while patients in the control group received a placebo. On day 6, approximately one-quarter (23.6%) of patients in the intervention group and one-third (31.6%) of patients in the placebo group tested negative for SARS-CoV-2 by RT-PCR. Although the differences were not statistically significant. Patients in the ivermectin group were all successfully discharged, while the placebo group had a 93% success rate. Mohan. et al. reported that the inclusion of ivermectin in the treatment regimens of patients with COVID-19 could not be definitively confirmed based on the results of this trial; because, aside from the slight advantage of successful patient discharge, no other advantages were observed (Mohan et al. 2021). Chee Loon Lim et al. designed another open-label, randomized clinical trial of 490 COVID-19 patients in various public hospitals in Malaysia public hospitals to investigate the effectiveness of ivermectin in preventing the progression of severe

disease in high-risk COVID-19 patients. The study patients received oral ivermectin (0.4 mg/kg body weight daily) for 5 days in addition to standard of care. The results showed that there was no significant difference between the groups. They reported that 21.6% of the ivermectin group progressed to serious disease and 17.3% of the control group (standard of care only) progressed to serious disease. Therefore, treatment with ivermectin in the early stages of COVID-19 could not prevent the disease from progressing to more serious stages (Lim et al. 2022a). Vallejos et al. reported the same results of their study, conducted on 501 people who were not hospitalized with COVID-19 in Corrientes, Argentina, between August 19, 2020 and February 22, 2021 (Vallejos et al. 2021).

A randomized, double-blind, single-point trial was conducted by LópezMedina et al. in Cali, Colombia to see the efficacy of ivermectin therapy in patients with moderate COVID-19. The participants were randomly selected from the electronic database of symptomatic, laboratory-identified patients with COVID-19 from the state health agency (July 15th to November 30th, 2020). Study participants received 300 g/kg.B.W of oral ivermectin in solution or an equivalent amount of placebo for 5 days. In the ivermectin group, the median symptom relief period was 10 days compared to 12 days in the placebo group. By day 21, 82% of patients taking ivermectin and 79% of patients taking placebo had no symptoms. The most commonly reported side effect is headache, which was reported by 52% of patients taking ivermectin and 56% of patients taking placebo. They concluded that using ivermectin for 5 days did not significantly improve symptom remission in people with moderate COVID-19. However, further trials may be needed to understand the effects of ivermectin on other clinically relevant outcomes (López-Medina et al. 2021). Samaha et al. in a randomized controlled study of 100 asymptomatic Lebanese COVID-19 patients investigated the beneficial effect of ivermectin in reducing the SARS-CoV-2 viral load. The difference between the participants' Ct values was not significant ($p = 0.06$) prior to ivermectin administration, suggesting that the participants' viral loads were the same. They were divided into two groups and one group took ivermectin. 72 h after the start of the diet, Ct values were significantly elevated in the ivermectin supplement group compared with the control group. In addition, the control group had more patients who developed clinical symptoms. They concluded that ivermectin appeared to provide therapeutic benefit, leading to fewer symptoms, lower viral loads, and fewer hospitalizations (Samaha et al. 2021). The mentioned clinical trials are summarized in Table 1.

Ivermectin potential prophylactic effects against COVID-19

In 18 randomized controlled trials of ivermectin for COVID-19, the drug was reported to play a statistically significant role in reducing viral clearance time, clinical recovery time, and mortality. Additionally, several controlled prophylaxis trials have shown that ivermectin can significantly reduce the risk of contracting COVID-19 (Kory et al. 2021). A recent study by Behera et al. found that oral administration of ivermectin in two doses (300 mcg/kg every 72 h) to healthcare workers as a chemopreventive agent reduced the risk of contracting COVID-19 by 83% over the next month (Behera et al. 2021). Therefore, it may be hypothesized that ivermectin can be used not only for the treatment of COVID-19, but also as a chemopreventive agent for high-risk occupational groups such as healthcare workers and high-risk groups for COVID-19 severity, such as patients with primary diseases such as immunity. Reduce your risk of contracting COVID-19 by eliminating deficiencies and malignancies. In a study by Okumush and colleagues, ivermectin could be an alternative or complementary option to current treatment protocols available to treat COVID-19. This could improve predictive laboratory parameters, accelerate clinical recovery, and reduce mortality even in severe COVID-19 patients.

Administration of ivermectin to patients without the MDR1/ABCB1 and/or CYP3A4 mutations is safe and not expected to cause serious side effects and has the potential to alleviate side effects with appropriate treatment (Okumuş et al. 2021). As one of the most widely distributed drugs in the body, ivermectin is rapidly absorbed by mouth, metabolized in the liver (cytochrome P450 system), and in healthy individuals binds strongly to plasma proteins such as albumin. In contrast, the severity of lung injury is associated with hypoalbuminemia in COVID-19 patients. Therefore, it was hypothesized that this observation could increase the availability of the free fraction of free plasma ivermectin. As one of the most widely distributed drugs in the body, ivermectin is rapidly absorbed by mouth, metabolized in the liver (cytochrome P450 system), and in healthy individuals binds strongly to plasma proteins such as albumin. In contrast, the severity of lung injury is associated with hypoalbuminemia in COVID-19 patients. Therefore, it was hypothesized that this observation could increase the availability of the free fraction of free plasma ivermectin (Canga et al. 2008; Klotz et al. 1990; Wu et al. 2021). Co-administration of ivermectin with antibiotics, antivirals, and corticosteroids may enhance effectiveness, avoid the need for high doses, and reduce the risk of toxicity and side effects in a dose-dependent manner. Therefore, ivermectin could be a promising safe treatment for COVID-19; ivermectin mechanisms against SARS-CoV-2 virus as a multifunction medication.

Table 1 Clinical trials investigated the anti-COVID-19 effects of ivermectin

	Number of patients	Country of study	Ivermectin dose	Description/outcome	References
Positive responded studies	280	United States	200 mg/kg/day	1) Reduced mortality in both general and severe lung disease patients compared to the control 2) No significant difference in length of hospitalization relative to the control	Rajter et al. (2021)
	69	Iran	0.2 mg/kg/day	Significantly reduced mean dyspnea, persistent cough, hospitalization, and incidence of lymphopenia	Shahbaznejad et al. (2021a)
	72	Bangladesh	12 mg	The safety and effectiveness of Ivermectin were confirmed as well as the effect of Ivermectin on the virus clearance rate and improvement of clinical symptoms	Ahmed et al. (2021)
	66	Turkey	200 µg/kg/day	Improved clinical recovery, improve prognostic test indicators, and reduce mortality	Okumuş et al. (2021)
Negative responded studies	125	India	12 and 24 mg/day	Increased negative RT-PCR tests or decreased viral loads in patients receiving Covid19, but no statistically significant difference compared with the placebo group	Mohan et al. (2021)
	490	Malaysia	0.4 mg/kg/day	No significant difference compared to the control, so treatment with Ivermectin in the early stages of COVID-19 could not prevent the disease from progressing to more serious stages	Lim et al. (2022b)
	501	Argentina	12 and 18 mg/day	No significant difference compared to the control	Vallejos et al. (2021)
	476	Colombia	300 g/kg/day	No significant improved symptom remission of COVID-19	López-Medina et al. (2021)
	100	Lebanon	9 mg, 12 mg, and 150 µg/kg	1) Significantly elevated Ct values compared with the control group 2) Fewer symptoms, lower viral loads, and fewer hospitalizations compared with the control group	Samaha et al. (2021)

Ivermectin is a drug with a wide range of biological activities. Initially used for multi-purpose veterinary applications, it has been used successfully to treat parasitic infections in humans for over 30 years (Omura 2008). Originally proposed in veterinary medicine and medicine for the treatment of onchocerciasis (Fodjo et al. 2019; Hopkins 2005; Otabil

et al. 2019), strongyloidiasis (Henriquez-Camacho et al. 2016; Igual-Adell et al. 2004), lymphatic filariasis (Beng et al. 2020; Brown et al. 2000; Kazura 1993), and scabies (Anderson and Strowd 2017; Rosumeck et al. 2018). It has now been demonstrated that ivermectin can be used to limit a variety of ailments, including orbital myopathy, trichinosis, malaria, leishmaniasis, African trypanosomiasis, asthma, epilepsy, neurological disorders, certain cancers, and a wide range of diseases caused by viruses (Li et al. 2021). There is also research information on the beneficial effects of ivermectin on a wide range of RNA and DNA viruses, including positive single-stranded RNA viruses such as ZIKV, Dengue virus, and Venezuelan Equine Encephalitis Virus (VEEV). Similarly, inhibition of nuclear entry of other viral proteins/genomes into host cells (Heidary and Gharebaghi 2020) stimulates the introduction of SARS-CoV-2 with similar genomic properties. In this regard, the antiviral effect of ivermectin on SARS-CoV-2 has now been published (Caly et al.). It has been shown that a single dose of ivermectin can reduce viral RNA approximately 5000-fold in Vero/hSLAM cells affected by SARS-CoV-2 48 h after treatment (Caly et al. 2020). However, these results are attracting considerable attention from researchers around the world. This conclusion should be drawn with caution as this study only investigated the antiviral effect of ivermectin on SARS-CoV-2 in vitro (Schmith et al. 2020).

One of the first in vivo studies to examine the effects of ivermectin on COVID-19 was Sabeena (Ahmed et al.). In this study, they gave patients ivermectin for 5 days and found that treated patients had significantly higher rates of viral clearance and significantly reduced disease severity indicators (CRP and LDH) compared to placebo (Kazura 1993). A meta-analysis also showed that ivermectin can reduce inpatient mortality by 68% (Chen et al. 2020). Given the antiviral effect of ivermectin, it has been suggested that this drug may directly or indirectly affect the pathogenesis of COVID-19 through inactivation of extracellular viral particles, interfering with the pathways of virus entry into the host cells, and viral replication, protein production, and post-translational changes and other probable pathways. The exact mechanism of action of this drug against COVID-19 disease has not yet been elucidated, but some mechanisms have been previously mentioned and are briefly described below.

Ivermectin in combination with other drugs

There are many published studies explaining the effectiveness of ivermectin in combination with other drugs. Previous studies have shown that the combination of ivermectin and doxycycline may reduce the recovery period and the proportion of patients who progress to advanced stages of the disease. In addition, this combination was negative for a COVID-19 test using RT-PCR on day 14 (Mahmud et al. 2021). Hashim et al. also concluded that treatment with the combination of ivermectin and doxycycline could reduce mortality in severely ill patients (Hashim et al. 2020). Butters' study found that the antiviral activity of ivermectin could be improved when combined with a zinc supplement. It has been suggested that increasing zinc levels may help fight infection in people with COVID-19 and speed up the recovery process (Butters and Whitehouse 2021).

Elalfy et al. explored the effects of a unique combination of ivermectin in mild-to-moderate cases of COVID-19 receiving treatment at home. Results showed that the combination of ivermectin with nitazoxanide, ribavirin, and zinc reduced the COVID-19 viral load in nasopharyngeal swabs. The synergistic effect of zinc in combination with antiviral agents has been demonstrated in other viral infections including hepatitis C virus, pediatric viral diarrhea, human papillomavirus, and human immunodeficiency virus. This study showed that 88% of patients treated with this combination had negative RT-PCR results on 15th of infection (Elalfy et al. 2021).

Clinical studies have shown that azithromycin inhibits the release of cytokines, attenuates the inflammatory response, and enhances the immunoglobulin response. Depending on its antiviral and anti-inflammatory properties, azithromycin alone may be an effective treatment for early COVID-19 (Andreani et al. 2020). The antiviral activity of azithromycin is attributed to several mechanisms, including structural and functional lysosomal damage to infected cells, inhibition of lysosomal proteases that promote the binding of SARS-CoV-2 to receptors such as ACE2, and viral entry into host cells (Al-Kuraishy et al. 2020). There was no clear interaction between ivermectin and azithromycin and no torsadogenic effect was observed (Al-Kuraishy et al. 2020). Therefore, the combination of azithromycin and ivermectin can be considered effective in COVID-19 patients.

Combinations of antiviral drugs with drugs that act on cellular targets or use a different mechanism of action also help to minimize drug resistance and toxicity during antiviral therapy (Day and Siu 2016). Several effective drug combinations are available for the treatment of HIV-1 and hepatitis C viruses (Ghany et al. 2019; Organization 2018). Remdesivir and ivermectin are two repurposed drugs that have received significant interest in the treatment of COVID-19. Remdesivir is a prodrug, a nucleotide analog, which acts against RNA viruses and inhibits RNA polymerase. It showed effective inhibitory activity against SARS-CoV-1 and Middle East Respiratory Syndrome (MERS-CoV) in vitro (Agostini et al. 2018; Sheahan et al. 2017). Additionally, Remdesivir was demonstrated to have antiviral efficacy against SARS-CoV-2 in vitro early in the epidemic and was subsequently clinically evaluated in humans (Pizzorno et al. 2020). Jeffreys et al. (Jeffreys et al. 2020) reported increased

antiviral activity against SARS-CoV-2 in vitro through a synergistic interaction between remdesivir and ivermectin. The combination of remdesivir and ivermectin may enhance the antiviral properties of remdesivir as a viral RNA polymerase inhibitor while facilitating the use of ivermectin's anti-inflammatory and/or immunomodulatory properties. However, more research is needed to evaluate the combined effects of ivermectin and remdesivir on COVID-19.

Favipiravir, an antiviral drug approved for use during the 2014 influenza pandemic in Japan, showed potent antiviral activity against SARS-CoV-2 in vitro. This drug has shown a wide range of therapeutic safety. According to the COVID-19 clinical study, it clears the viral infection faster than lopinavir/ritonavir (LPV/RTV) and recovers faster than umifenovir. In the end, favipiravir was proven effective in clinical trials in China, Russia, and Japan. In addition, clinical trials are underway in several countries, including the United States, United Kingdom, and India (Joshi et al. 2021a, b). Recent studies have identified favipiravir and ivermectin as a promising drug combination for clinical trial testing for the treatment of COVID-19 due to their synergistic effects, a fairly high safety profile, and easy availability (Jitobaom et al. 2021).

Ribavirin, an analog of guanosine, has significant antiviral activity against DNA and RNA viruses. Although the exact mechanism of action of ribavirin has not been elucidated, one of the possible mechanisms is the inhibition of mRNA capping and the induction of mutations during viral replication. Such mechanisms can limit viral proliferation (Crotty et al. 2001; Graci and Cameron 2006). Researchers have investigated ribavirin as a potentially effective antiviral agent against SARS-CoV2 infection based on previous clinical experience with SARS-CoV-2 and Middle East Respiratory Syndrome (MERS) coronaviruses (Al-Tawfiq et al. 2014; Booth et al. 2003; Elalfy et al. 2021). After ribavirin was recommended by the Chinese government for the treatment of SARS-CoV-2 infection, numerous clinical trials have been conducted to evaluate its effectiveness in treating SARS-CoV-2 infection (Hung et al. 2020). Studies have shown that ribavirin is more effective in treating COVID-19 when combined with interferon-α or lopinavir/ritonavir (Yousefi et al. 2020; Zhong et al. 2020). However, in studies using only ribavirin, its efficacy was reduced compared to the control drug (Elalfy et al. 2021). As a result, ribavirin monotherapy has a limited therapeutic effect on COVID-19, and the dose must be increased to enhance the effect. On the other hand, the increase in dose may lead to side effects such as hepatotoxicity and Hematological problems (Sanders et al. 2020).

Elalfy et al. found that co-administration of ivermectin, ribavirin, and nitazoxanide with zinc supplementation cleared SARS-CoV-2 in the nasopharynx significantly faster than symptomatic therapy (Elalfy et al. 2021). Therefore, it can be concluded that the combination therapy of ivermectin and ribavirin can be an effective treatment for COVID-19 with a synergistic effect and a lower risk of dose-dependent side effects compared to ivermectin monotherapy. At the same time, further studies are needed to confirm the combined effects of these drugs.

Famotidine, as a histamine-2 receptor antagonist, inhibits the action of histamine in parietal cells, ultimately inhibiting gastric acid secretion (Freedberg et al. 2020). It was previously reported to inhibit HIV.1 replication in vitro (Bourinbaiar and Fruhstorfer 1996). A recent study in the United States reported that famotidine helped improve the clinical outcome of 1620 inpatients with COVID-19 and reduce the risk of death (Freedberg et al. 2020). Based on the data of Sen Gupta et al. (Sen Gupta and Rana 2020), it was suggested that the combination of famotidine and ivermectin may have a synergistic effect. As a first-level barrier, famotidine inhibits viral entry. Ivermectin then acts as a second barrier, preventing virus replication in the host cell, thereby completely inhibiting the virus (Sen Gupta and Rana 2020). In addition, these drugs are more attractive because of their safety, availability, and cost effectiveness. However, it is suggested that additional studies are needed to confirm the synergistic effect of ivermectin and famotidine combination therapy as a promising treatment for COVID-19.

Ivermectin against future variants

Because the SARS-CoV-2 vaccine targets the biology of the spike protein, there is growing concern about the recently documented "hard-to-vaccine strain". In these circumstances, ivermectin may work with the strategies mentioned for these new strains that can evade immunity with a vaccine (Zaidi and Dehgani-Mobaraki 2021), such as Delta, the newly emerged Omicron variant, and possibly future supervariants. However, new studies are needed to evaluate the efficacy of ivermectin against emerging variants that bind to the SARS-CoV-2 spike protein and block its binding to established host cell receptors including ACE2, CD147, neuropilin-1, etc. (Zalpoor et al. 2022c).

Omicron contains a variety of mutations. There are at least 32 mutations within its spike protein alone in comparison to the Delta variant. In addition to these newly discovered mutations, the Omicron variant is dependent on NSP12 for viral replication and NSP14 for methyltransferase and exoribonuclease activity. Therefore, they can have a positive effect on viral RNA replication. It is thought that the Omicron variant is at least three times more infectious in comparison to the original SARS-CoV-2 and possibly even more infectious than the delta variant (Raj 2021; Saxena et al. 2022). On the one hand, ivermectin binds to the predicted

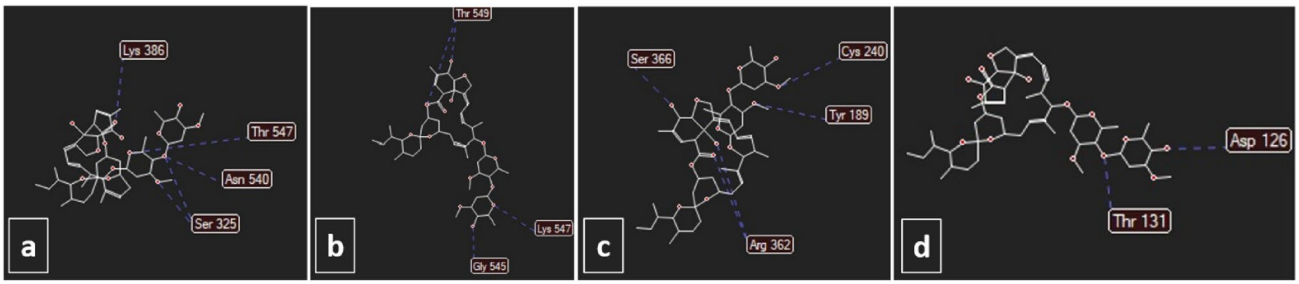

Fig. 3 Docking of **a** spike Delta (score: 120), **b** spike Omicron (score: 124), **c** RdRp (score: 137), and **d** NSP14 (score: 147) with ivermectin. The unit of scoring function is moldockscore. The energies were obtained using molegro virtual docker

active site of NSP14 and RNA-dependent RNA polymerase (RdRp) with a high affinity (Zaidi and Dehgani-Mobaraki 2021) which are highly required for viral replication. On the other hand, we postulate that ivermectin possibly may bind to the Spike protein of Omicron, as seen in previous variants. For Assessment of these effects of ivermectin, we used molegro virtual docker to simulate ivermectin potential for binding to the active site of NSP14, RdRp, TMPRSS2, Delta, and Omicron variant spike protein, which we found that not only ivermectin can bind to these proteins with high affinity but also it binds to Omicron spike protein with higher affinity than Delta spike protein (Fig. 3).

Conclusion and future directions

Ivermectin alone or in combination with other drugs appears to have beneficial effects on SARS-CoV-2 infection. In addition, ivermectin is known to inhibit the entry and replication of viral RNA into host cells through a variety of strategies/pathways. However, we hypothesize that ivermectin is capable of increasing the replication capacity of SARS-CoV-2 by enhancing autophagy. Although ivermectin has anti-inflammatory properties, it can induce pathological complications and inflammatory responses during COVID-19 treatment by increasing stimulation of the P2X7 receptor and its downstream signaling pathways. Furthermore, despite its role in altering metabolic processes, it may induce ROS and it may be one of the other side effects of ivermectin in the treatment of COVID-19. It appears that the combined treatment of ivermectin with other drugs may reduce side effects and demonstrate its therapeutic efficacy in the treatment of COVID-19.

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Author contributions The core idea of this study came from MNA. MNA, FM, HZ, FA, AA, HE, HMS, and AM. M and HE wrote the manuscript. MNA, HZ, HMS, and EB edited the manuscript. FM created the figures. FA created the docking. All authors approved the manuscript and they have declared that no conflict of interest exists.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest All other authors declare no conflict of interest.

Ethical approval There is no involvement of human or animal in this study.

Consent for publication All other authors declare no conflict of interest.

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