REVIEW

Ivermectin as a potential drug for treatment of COVID-19: an in-sync review with clinical and computational attributes

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Abstract

Introduction COVID-19 cases are on surge; however, there is no efficient treatment or vaccine that can be used for its management. Numerous clinical trials are being reviewed for use of different drugs, biologics, and vaccines in COVID-19. A much empirical approach will be to repurpose existing drugs for which pharmacokinetic and safety data are available, because this will facilitate the process of drug development. The article discusses the evidence available for the use of Ivermectin, an anti-parasitic drug with antiviral properties, in COVID-19.

Methods A rational review of the drugs was carried out utilizing their clinically significant attributes. A more thorough understanding was met by virtual embodiment of the drug structure and realizable viral targets using artificial intelligence (AI)-based and molecular dynamics (MD)-simulation-based study.

Conclusion Certain studies have highlighted the significance of ivermectin in COVID-19; however, it requires evidences from more Randomised Controlled Trials (RCTs) and dose- response studies to support its use. In silico-based analysis of ivermectin's molecular interaction specificity using AI and classical mechanics simulation-based methods indicates positive interaction of ivermectin with viral protein targets, which is leading for SARS-CoV 2 N-protein NTD (nucleocapsid protein N-terminal domain).

Keywords Ivermectin · COVID-19 · SARS-CoV-2 · Treatment

Abbreviations

SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
COVID-19	Coronavirus disease 2019

Introduction

Numerous clinical trials are being reviewed for use of multiple drugs, biologics, and vaccines in COVID-19. Ivermectin is one of the potential drugs that can be repurposed for use against SARS-CoV-2 infection. As of 16-10-2020, 38 Ivermectin trials are registered with ClinicalTrials.gov and 8 with Clinical Trials Registry, India (CTRI) to validate its use in treatment of COVID-19. Table 1 provides details of clinical trials of ivermectin.

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Ivermectin belongs to class 'avermectins' consisting of 16-membered macrocyclic lactone compounds [1]. It is approved by FDA for use as an anti-parasitic drug [2] and is known to have nematocidal, insecticidal, and acaracidal properties. Ivermectin was discovered in Japanese Kitasato Institute in the year 1967 and first got approval in 1987 for treatment of onchocerciasis (river blindness) caused by *Onchocerca volvulus* and transmitted by blackfly in humans. It is efficacious in filarial infections and eradicates parasites of gastrointestinal tract. It is also used for treatment of malaria, trypanosomiasis, head lice, scabies, and leishmaniasis [1]. Moreover, it also exhibits antibacterial and anticancer activities [3]. Ivermectin is safe at higher doses and frequent regimens. Guzzo et al. showed that higher doses of ivermectin 120 mg (up to 2,000 µg/kg) taken once or at 180 mg (up to 3,000 µg/kg) taken in split doses over 1 week is well-tolerated and safe [4]. Furthermore, ivermectin has shown antiviral activity against various RNA as well as DNA viruses [5] and is now being evaluated for use in COVID-19. Moreover, it can be used in cases, where use of hydroxychloroquine is not feasible. Hydroxychloroquine in COVID-19 is limited in some cases, because

Table 1 Clinical trials of ivermectin (from ClinicalTrials.gov and CTRI as of 16-10-2020)

	Trial registration	Phase/status	Intervention/comparator	Study design	Size/location
1	NCT04343092	Phase 1 Completed	Ivermectin	Randomized, parallel Masking: double	100 Iraq
2	NCT04422561	Phase 2/phase 3 Completed	Ivermectin	Randomized, sequential Masking: none	340 Egypt
3	NCT04434144	Completed	Ivermectin + doxycycline Hydroxychloroquine + azithromycin	Prospective, case-only	116 Bangladesh
4	NCT04381884	Phase 2 Completed	Ivermectin plus standard care Control arm will receive standard care	Randomized, parallel Masking: none	45 Argentina
5	NCT04446104	Phase 3 Completed	Hydroxychloroquine sulfate tablets Ivermectin 3 Mg Tab Zinc Povidone-iodine Supplement: vitamin C	Randomized, parallel Masking: none	4257 Singapore
6	NCT04523831	Phase 3 Completed	Ivermectin and doxycycline Standard of care	Randomized, parallel Masking: double	400 Bangladesh
7	NCT04438850	Phase 2 Recruiting	Ivermectin Placebo	Randomized, sequential Masking: quadruple	102 Italy
8	NCT04425707	Not applicable Recruiting	Ivermectin	Randomized, parallel Masking: none	100 Egypt
9	NCT04429711	Not applicable Recruiting	Ivermectin oral product	Randomized, parallel Masking: quadruple	100 Israel
10	NCT04405843	Phase 2| Phase 3 Recruiting	Ivermectin oral product Placebo	Randomized, parallel Masking: quadruple	400 Colombia
11	NCT04445311	Phase 2|Phase 3 Recruiting	Ivermectin	Randomized, parallel Masking: none	100 Egypt
12	NCT04392713	Not applicable Recruiting	Ivermectin 6 MG oral tablet (2 tablets)	Randomized, parallel Masking: none	100 Pakistan
13	NCT04351347	Phase 2|Phase 3 Recruiting	Ivermectin Nitazoxanide with ivermectin Ivermectin wth chloroquine	Randomized, parallel Masking: none	300 Egypt
14	NCT04431466	Phase 2 Recruiting	Ivermectin Standard treatment for COVID-19	Randomized, parallel Masking: triple	64 Brazil
15	NCT04529525	Phase 2|Phase 3 Recruiting	Ivermectin Placebo	Randomized, parallel Masking: quadruple	500 Colombia
16	NCT04384458	Not applicable Recruiting	Hydroxychloroquine Ivermectin	Randomized, parallel Masking: none	400 Brazil
17	NCT04373824	Not applicable Recruiting	Ivermectin	Non-randomized, crossover Masking: None	50 India
18	NCT04403555	Phase 2|Phase 3 Recruiting	Ivermectin Doxycycline Chloroquine	Randomized, parallel Masking: None	200 Egypt
19	NCT04447235	Phase 2 Recruiting	Placebo Ivermectin Losartan	Randomized, parallel Masking: double	176 Brazil
20	NCT04472585	Phase 1|Phase 2 Recruiting	Nigella sativa/black cumin Ivermectin injectable solution Placebo Zinc	Randomized, parallel Masking: quadruple	40 Pakistan
21	NCT04399746	Not applicable Recruiting	Ivermectin Azithromycin Cholecalciferol	Non-randomized, parallel Masking: none	30 Mexico

Table 1 (continued)

	Trial registration	Phase/status	Intervention/comparator	Study design	Size/location
22	NCT04374019	Phase 2 Recruiting	Hydroxchloroquine and azithromycin Ivermectin Camostat mesilate Artemesia annua	Randomized, parallel Masking: none	240 US
23	NCT04391127	Phase 3 Active, not recruiting	Hydroxychloroquine Ivermectin Placebo	Randomized, parallel Masking: double	108 Mexico
24	NCT04390022	Phase 2 Active, not recruiting	Ivermectin Placebo	Randomized, parallel Masking: double	24 Spain
25	NCT04425863	Active, not recruiting	Ivermectin 5 mg/mL	Prospective, cohort	100 Argentina
26	NCT04425850	Active, not recruiting	Iota carrageenan Ivermectin	Prospective, cohort	70 Argentina
27	NCT04407130	Phase 2 Enrolling by invitation	Ivermectin + doxycycline + placebo Ivermectin + placebo Placebo	Randomized, parallel Masking: double	72 Bangladesh
28	NCT04510233	Phase 2 Not yet recruiting	Ivermectin nasal Ivermectin oral Standard care	Randomized, parallel Masking: none	60
29	NCT04360356	Phase 2| Phase 3 Not yet recruiting	Ivermectin plus Nitazoxanide Standard Care	Randomized, parallel Masking: double	100
30	NCT04407507	Phase 2 Not yet recruiting	Ivermectin Placebo	Randomized, parallel Masking: single	66
31	NCT04392427	Phase 3 Not yet recruiting	Nitazoxanide, ribavirin and ivermectin for 7 days	Randomized, sequential Masking: single	100 Egypt
32	NCT04435587	Phase 4 Not yet recruiting	Ivermectin pill Combined ART/hydroxychloroquine	Randomized, parallel Masking: single	80 Thailand
33	NCT04382846	Phase 3 Not yet recruiting	Nitazoxanide Ivermectin Chloroquine Azithromycin	Randomized, parallel Masking: none	80
34	NCT04460547	Not yet recruiting	Convalescent plasma transfusion Hydroxychloroquine DAS181 Ivermectin Interferon beta-1A	Retrospective, cohort	200
35	NCT04482686	Phase 2 Not yet recruiting	Ivermectin Doxycycline Hcl Zinc Vitamin D3 Vitamin C	Randomized, parallel Masking: triple	300 US
36	NCT04551755	Phase 2 Not yet recruiting	Ivermectin and doxycycline Placebo	Randomized, parallel Masking: triple	188
37	NCT04530474	Phase 3 Not yet recruiting	Ivermectin pill Placebo	Randomized, parallel Masking: triple	200 US
38	NCT04527211	Phase 3 Not yet recruiting	Ivermectin	Randomized, parallel Masking: quadruple	550 Argentina
39	CTRI/2020/04/024858	Not yet recruiting	Ivermectin (200–400 mcg/kg on day 1 and 2 in addition to standard treatment) Standard treatment	Non-randomized, active controlled	50 New Delhi, India

Table 1 (continued)

	Trial registration	Phase/status	Intervention/comparator	Study design	Size/location
40	CTRI/2020/04/024948	Phase 2 Not yet recruiting	Ciclesonide (200 mcg twice a day for 7 days) Hydroxychloroquine (400 mg twice a day, Day1 followed by 200 mg twice a day on Days 2–7) Ivermectin (12 mg once a day for 7 days) Standard of care	Randomized, parallel	120 New Delhi, India
41	CTRI/2020/05/025224	Phase 2 Not yet recruiting	Ivermectin (12 mg once a day at night, oral for 2 days with standard of care) Standard of care	Randomized, parallel	50 Madhya Pradesh, India
42	CTRI/2020/06/025960	Not yet recruiting	Ivermectin (12 mg, per orally, once a day for 3 days) Standard of care	Randomized, parallel, active controlled	100 Maharashtra, India
43	CTRI/2020/06/026232	Phase 3 Not yet recruiting	Ivermectin (single oral dose of 200 mcg/kg)	Single arm	50 Andhra Pradesh, India
44	CTRI/2020/08/027225	Not yet recruiting	Ivermectin (12 mg orally on days 1 and 2) Placebo tablets	Randomized, parallel, placebo controlled	90 Bihar, India
45	CTRI/2020/08/027282	Phase 3 Not yet recruiting	Ivermectin 12 mg or 36 mg one dose orally one time a day (two intervention arms) Two multivitamin tablets	Randomized, parallel, multiple arm	180 Uttar Pradesh, India
46	CTRI/2020/09/027944	Phase 3 Not yet recruiting	Cefixime 200 mg (BD, 5 days), Ivermectin 12 mg (OD, day 1), Montelukast 10 mg (OD, 5 days), Ascoril LS 5 ml (TID, 5 days) Cefixime 200 mg, vitamin C, MVBC, antacids	Randomized, parallel group, active controlled	30 Maharashtra, India

it can occasionally cause QTc prolongation and effective antiviral tissue levels need 5–10 days to accrue at maximum safe daily dosage [6]. Ivermectin is not found to be associated with such side effects and treatment with it can also be more cost-effective [7]. Following is an in tuned study about the clinical and molecular attributes of ivermectin.

Mechanism of action of ivermectin

Ivermectin enhances the activity of GABA receptors or glutamate-gated chloride ion channels in parasites and helminths which blocks the signal between neuron and muscle. GABA sensitive neurons of mammals are protected by blood brain barrier (BBB), and thus protect vertebrates from possible adverse effects of ivermectin. However, invertebrates are dose-dependently susceptible because of extensive distribution of chloride ion channels, where ivermectin generates Cl^- influx, resulting in hyperpolarization which impedes myosin II light chain's phosphorylation. This promotes paralysis of somatic muscles with concomitant uncoordinated movement, starvation because pharyngeal pumping is inhibited, and death. Ivermectin's affinity for parasite is 100 times more than for brain of mammals [1]. Immunomodulation of host response is another mechanism by which ivermectin can act achieved by activation of neutrophils, increased C-reactive protein and interleukin-6 levels [8]. For its antiviral activity, ivermectin is believed to act through inhibition of nuclear import of proteins of virus as well as of host. Majority of the RNA virus depend on IMPα/β1 at the time of infection, and ivermectin inhibits this import and enhances the antiviral response [9]. Another mechanism of action by which ivermectin is believed to act involves transmembrane receptor CD147. CD147 along with ACE-2 has been recognized as a key binding site for SARS-CoV-2 spike protein. The potential for major dose–response gains is assessed on the basis of studies that indicate that ivermectin shields SARS-CoV-2 spike protein which binds to CD147 and ACE-2 [6]. Furthermore, Rizzo suggested that ivermectin may have a possible ionophore role. Ionophores have been appreciated for their antibiotic activity

and their role as antiviral and anticancer agents is also hypothesized [10].

Another, mechanism of action of ivermectin which needs consideration involves the allosteric modulation of the P2X$_4$ receptor. P2X receptors are the channels selective to cation and are gated by extracellular ATP [11]. They mediate a number of functions in health and disease through extracellular ATP [12, p. 4]. From the seven subunits of P2X receptors, P2X$_4$ is most sensitive to ivermectin. Priel et al. studied the effect of ivermectin on whole cell as well as single channel currents of P2X$_4$ receptors of humans expressed in HEK293 cells. Authors observed that at low ivermectin concentrations maximal current activated by ATP is predominantly activated and at high concentrations rate of current deactivation is predominantly slowed and potency of ATP is enhanced. Hence, ivermectin possibly binds to different extracellular sites (higher and lower affinity sites) on the receptor and modulate the amplitude of current and rate of deactivation of current [11]. Positive allosteric modulation of P2X$_4$ by ivermectin enhances ATP-mediated secretion of CXCL5 (pro-inflammatory chemokine). CXCL5 is a chemo-attractant molecule expressed in inflammatory cells in different tissues and modulates neutrophil chemotaxis and chemokine scavenging [13]. Furthermore, ivermectin (2 mg/kg) was shown to have anti-inflammatory effects in animal model of allergic asthma. Immune cell recruitment, cytokine production in broncho-alveolar lavage fluid, IgE and IgG1 secretion in serum as well as hyper-secretion of mucus by goblet cells was reduced significantly by ivermectin [14]. Moreover, ivermectin blocked inflammatory cytokine production induced by LPS in mice. Production of IL-6, IL-1ss, and TNF-α was reduced considerably both in vitro and in vivo and LPS induced translocation of NF-κB was curbed too [15]. Quantitative proteomics study by Li et al. had revealed broad-spectrum antiviral property of ivermectin which can be of use in treatment of COVID-19 [16].

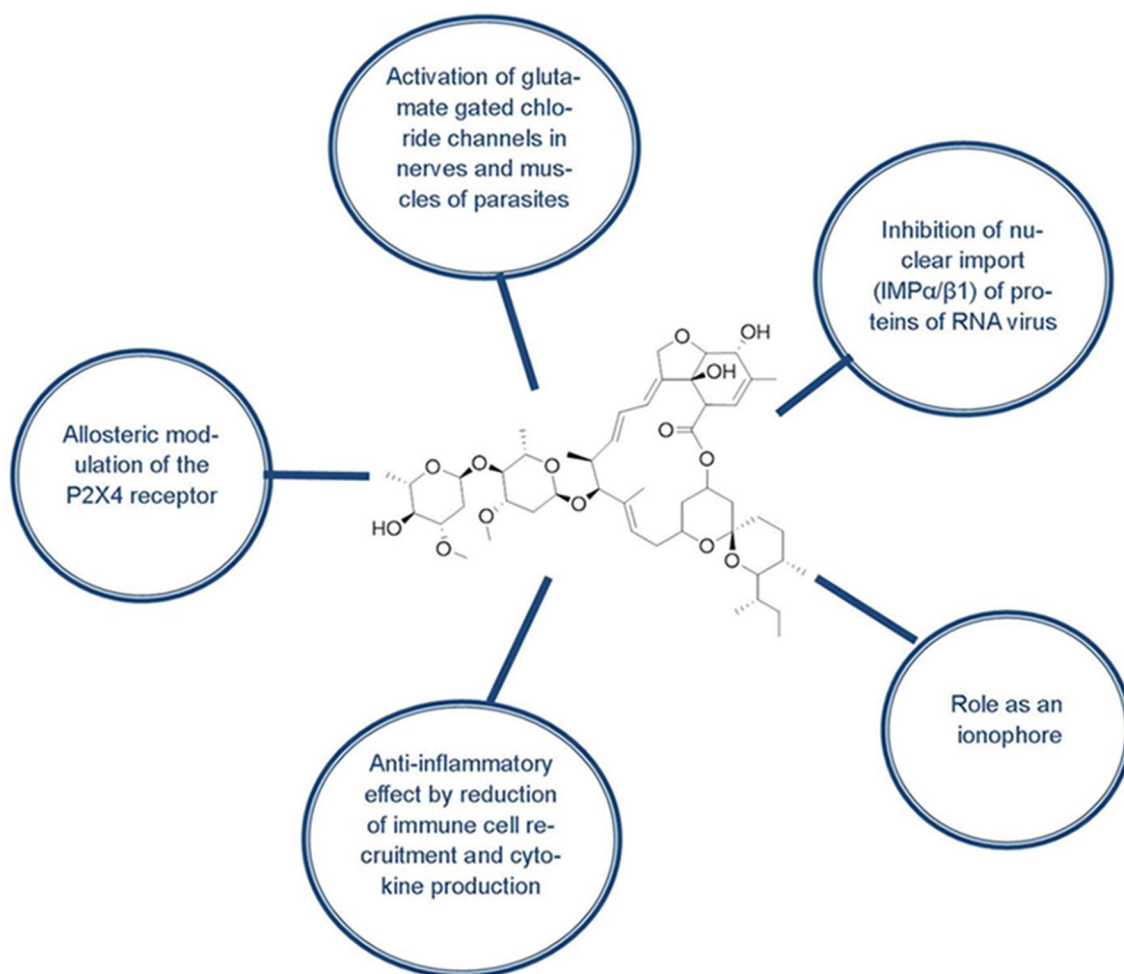Figure 1 describes possible mechanism of actions of ivermectin.

Fig. 1 Mechanism of actions of ivermectin

Evidence available for use of Ivermectin in COVID-19

In silico, in vitro as well as clinical studies have been carried out to check the efficacy of ivermectin against SARS-CoV-2 infection and are summarized in the subsequent paragraphs.

In silico

Abdel-Mottaleb et al. reported that ivermectin, hydroxychloroquine and favipiravir are the strongest binding drugs to ACE-2 as well as S protein [17] and molecular modeling study by Dayer demonstrated that ivermectin is one of the most efficient agent that shields SARS-CoV-2 spike protein from host cell receptors [18]. According to another study by Lehrer et al. ivermectin docked in the region of leucine 91 of the spike and histidine 378 of the ACE2 receptor. Furthermore, a study by Daghir Janabi reported high binding affinity of ivermectin to RNA dependent RNA polymerase (RdRp) [19].

In vitro

Caly et al. showed that when 5 µM of ivermectin was added to Vero/hSLAM cells with SARS-CoV-2 isolate Australia/VIC01/2020, viral RNA in the supernatant (indicated virions that were released) was reduced by 93% and RNA of virus associated with cell was reduced by 99.8% (indicated virions that were not released and packaged). Furthermore, it was stated that by 48 h ivermectin brought about 5000 fold reduction of viral RNA and the IC50 was found out to be ~ 2 µM [2].

Clinical

Rajter et al. carried out a retrospective cohort study ($n = 280$) of patients confirmed with SARS-CoV-2 infection hospitalized at a hospital in South Florida. They reviewed 173 patients who received treatment with ivermectin (at least one dose of ivermectin 200 mcg/kg orally along with usual clinical care) and 107 who received usual care and found out that treatment with ivermectin was related to lower mortality particularly in patients needing higher inspired oxygen or ventilator support. [20]. Another study by Alam et al. reported that ivermectin and doxycycline's combination is very efficacious in SARS-CoV-2 clearance in patients with mild to moderate disease. In their observational/cross-sectional study, they included 100 mild and moderate RT-PCR confirmed COVID-19 patients from Bangladesh. They were treated with combination of ivermectin (0.2 mg/kg single dose) and doxycycline (100 mg daily for 10 days) in addition to supportive treatment. Symptoms of all the patients improved within 72 h, no side effects were observed, intensive care admission was not required, no deaths were reported, and all of them tested negative [21]. Furthermore, Gorial et al. conducted a pilot clinical trial to evaluate the efficacy of ivermectin as additional treatment to hydroxychloroquine and azithromycin in mild to moderate hospitalized COVID-19 patients. 16 patients who were given ivermectin (200 mcg/kg on day of admission) as additional treatment to hydroxychloroquine and azithromycin were compared to control group ($n = 71$) who were given hydroxychloroquine and azithromycin. Cure rate was 100% in case of ivermectin group and 97.2% (69 out of 71 patients) in case of control group. Also the mean time to stay in the hospital was considerable less for the ivermectin group. No side effects were seen [22]. In another prospective comparative study by Rahman et al. ($n = 400$; patients with mild to moderate disease), effect of ivermectin in combination with doxycycline was compared to hydroxychloroquine in combination with azithromycin. 200 patients were administered ivermectin (18 mg on day 1) and doxycycline (100 mg two times a day for 5 days), whereas the another 200 were administered hydroxychloroquine (800 mg on day 1 and after that 400 mg every day for 10 days) and azithromycin (500 mg on day 1 and after that 250 mg every day for 4 days). According to the results ivermectin combined with doxycycline was safe and efficacious in early viral clearance in patients with mild to moderate disease and took less time than hydroxychloroquine and azithromycin combination for viral clearance [23]. Chowdhury et al. also compared combination of ivermectin and doxycycline to hydroxychloroquine and azithromycin in mild to moderate COVID-19 patients. Patients were categorized into 2 groups. The first group ($n = 60$) received ivermectin (200 mcg/kg one dose) and doxycycline (100 mg two times a day for 10 days) and the second ($n = 56$) received hydroxychloroquine (400 mg on day 1 and after that 200 mg two times a day for 9 days) and azithromycin (500 mg every day for 5 days). According to the study, ivermectin and doxycycline were superior to hydroxychloroquine and azithromycin in mild to moderate COVID-19 patients but the variation in time to become symptom free and time to negative PCR was not significant statistically [24]. Furthermore, Wijaya and Salim reported that there was significant clinical and radiological improvement in 3 confirmed COVID-19 patients after one dose of ivermectin [25]. In a cross-sectional study by Malik et al., majority of health care professionals were treated with ivermectin either in combination with azithromycin or with doxycycline and favorable outcomes were observed [26].

Table 2 Clinical efficacy and safety of ivermectin

References	Population	Intervention	Control	Outcome of intervention	Outcome of control	Adverse Event in intervention arm	Adverse Event in control arm
Rajter et al. [20]	280 COVID-19 patients	Ivermectin (at least one oral dose of ivermectin 200 mcg/kg) along with usual clinical care $N = 173$	Usual care $N = 107$	Overall mortality—15.0% Mortality in patients with severe illness—38.8%	Overall mortality—25.2% Mortality in patients with severe illness—80.7%	–	–
Alam et al. [21]	100 RT-PCR confirmed COVID-19 patients with mild to moderate disease	Ivermectin (0.2 mg/kg one dose) and doxycycline (100 mg every day for 10 days) in addition to supportive treatment	–	Symptoms of all the patients improved within 72 h, no side effects were observed, intensive care admission was not required, no deaths were reported, and all of them tested negative	–	–	–
Gorial et al. [22]	87 mild to moderate COVID-19 diagnosed patients	16 patients were given ivermectin (200 mcg/kg on day of admission) in addition to hydroxychloroquine and azithromycin	71 patients were given hydroxychloroquine and azithromycin	Cure rate—100% Mean time to stay in the hospital—7.62 ± 2.75 days	Cure rate—97.2% (69 out of 71 patients) Mean time to stay in the hospital—13.22 ± 5.90 days	–	–
Rahman et al. [23]	400 mild to moderate COVID-19 patients	Ivermectin (18 mg on day 1) and doxycycline (100 mg two times a day for 5 days) $N = 200$	Hydroxychloroquine (800 mg on day 1 and after that 400 mg every day for 10 days) and azithromycin (500 mg on day 1 and after that 250 mg every day for 4 days) $N = 200$	66% viral clearance at day 5 and 83.5% at day 6. 16.5% remained PCR positive after 6th day of taking Ivermectin	77.0% viral clearance at day 11 and 81.5% at day 12 of taking hydroxychloroquine. 18.5% remained PCR positive after day 12	Anorexia (23.5%), diarrhea (12%), skin rash (10%)	Anorexia (31%), diarrhea (7%), Skin rash (1%)

Table 2 (continued)

References	Population	Intervention	Control	Outcome of intervention	Outcome of control	Adverse Event in intervention arm	Adverse Event in control arm
Chowdhury et al. [24]	COVID-19 patients with mild to moderate illness	Ivermectin (200 mcg/kg one dose) and doxycycline (100 mg two times a day for 10 days) N=60	Hydroxychloroquine (400 mg on day 1 and after that 200 mg two times a day for 9 days) and azithromycin (500 mg every day for 5 days) N=56	All patients of reached negative PCR at 8.93 days (mean), symptomatic recovery, at 5.93 days (mean)	96.36% reached a negative PCR at 6.99 days (mean) and were having no symptoms at 9.33 days	Seen in 31.67% patients (comprising lethargy: 23.3%; nausea: 18.3%; and infrequent vertigo: 11.66%)	Seen in 46.43% (comprising mild blurring of vision and headache: 23.21%; enhanced lethargy and dizziness: 39.2%; infrequent palpitation: 17.85%; nausea and vomiting: 16.07%)

Table 3 Binding score of ivermectin against SARS-CoV-2 proteins

	Model	CNN BindingDB score
1	3CL protease	9.48
2	RNA dependent RNA polymerase	9.32
3	Helicase	9.34
4	Nucleocapsid	9.41

*Table 2 provides data on clinical efficacy and safety of ivermectin.

Artificial intelligence-based study

The Drug–Target Interaction (DTI) may be predicted by Artificial Intelligence using Deep Learning methods. This has been done by DeepPurpose, a Deep learning framework for drug discovery [27]. The drugs as well as the protein targets have to be encoded to formats appropriate for model creation. The drug (Ivermectin) and the protein targets were encoded into convolutional neural network (CNN) format [28]. Prediction of DTI was made against a model trained on drug target interactions available in the BindingDB database which consists of 13,349 drugs, 1,658 proteins and 74,641 interactions [29]. The pretrained model was available through the DeepPurpose framework (MSE of 0.600 and Concordance Index of 0.857). Analyzing the interaction of Ivermectin with SARS-CoV-2 proteins through Artificial Intelligence based DTI framework predicted a binding score of 9.48 against 3CL protease, 9.32 against RNA dependent-RNA polymerase, 9.34 against Helicase and 9.41 against nucleocapsid protein.

Table 3 provides binding score of ivermectin against SARS-CoV-2 proteins. Model was trained on BindingDB and both drug and protein sequence was encoded in CNN.

Molecular dynamics simulations-based study

Molecular dynamics simulations can be applied to analyze and conclude a framework of molecular level of microbial pathogenesis, but they are still open for improvements in algorithmic precision and methodologies that can effectively assess the bio-system of topics. In this study the molecular interactions of the ivermectin with some primary SARS-CoV-2 protein targets were simulated, namely with nucleocapsid protein N-terminal domain (6M3M), spike S1 RBD (PDB: 6M17), spike S2 fusion domain (6VXX), CL protease (6Y2F), nsp7, nsp8, nsp 12 and nsp13 (6XEZ)

as distinct components of RDRP protein and lastly ORF6 protein (I-tasser model) [30–32]. The proteins were optimized and simulated at physiological conditions (pH 7.4, Temp = 310 K, Press = 1.01325 Bar). All the simulations were performed on Desmond on Dell Precision tower 3630 with Quadro RTX 4000 GPU computing [33].

The idea that can be obtained from the molecular interaction profile of ivermectin with selected viral proteins is that the ivermectin shows a distinction between the degree of interaction specificity among the various viral targets, but still exhibits comparable binding profile with some. The Cα-RMSD for the 100 ns MD simulation shows the variation in average conformation change influenced by ivermectin on the target proteins (Fig. 2). The extremely smaller protein structures (nsp7, ORF6) encountered higher deviations in overall conformation and opposite implies for the bigger protein (S2 fusion domain). The residue interaction index and trajectory visualization add more to the information about the nature of interaction. It can be deduced that Ivermectin has efficient binding with: (1) spike S1-RBD, where it binds with Thr 500, Asn 501 and Tyr 505 residues (Fig. 3b). These sites are critical to the SARS CoV-2 spike protein-mediated recognition from ACE-2 receptors in the host cellular system. Prominent H-bonding with Thr 500 and Asn 501 and water bridges were observed for more than 80% of simulation (Fig. 4a). (2) Spike S2-fusion domain, it binds to two specific regions of S2 fusion domain namely HR-1

sub-domain and fusion peptide domain. Major interactions were observed at the HR-1 domain, where it binds for up to 80% of simulation duration with Ser 968, Asn 969 and Gly 971 with H-bonds and water bridges. The fusion peptide region also exhibits weak affinity for ivermectin at Phe 797 and extremely weak interactions at Pro 792 residues with hydrophobic contact (Figs. 3c, 4b). The S2 fusion domain is necessary to build the fusion bridge between the viral and host membrane, where the fusion peptide is highly non-polar flexible region which facilitates the direct contact with the host membrane components. (3) N-protein, the poly-nucleotide (RNA) interacting cleft of nucleocapsid N-protein characterized by residues Arg 69, Tyr 124, Asn 127 and Glu 137 were found interacting with ivermectin with rich H-bond ratio, see Figs. 3a and 4c. (4) Main protease, the main protease of the SARS-CoV 2 is another target which exhibits good affinity for ivermectin in inhibition site too at Glu 19, Thr 25, Glu 47, Leu 50 (Figs. 3d, 4d). The spatial localization of ivermectin molecule on the protein surface is illustrated with the active residue characterization in Fig. 5. On the contrary, RDRP components (nsp7, nsp8, nsp12) with helicase (nsp13) and the ORF-6 fragment had weak specificity for ivermectin and could be characterized as weak targets for ivermectin as there was significantly low number of observed drug-protein collisions in simulation.

The broad specificity of the ivermectin to these proteins and other reported pharmacological targets can be attributed
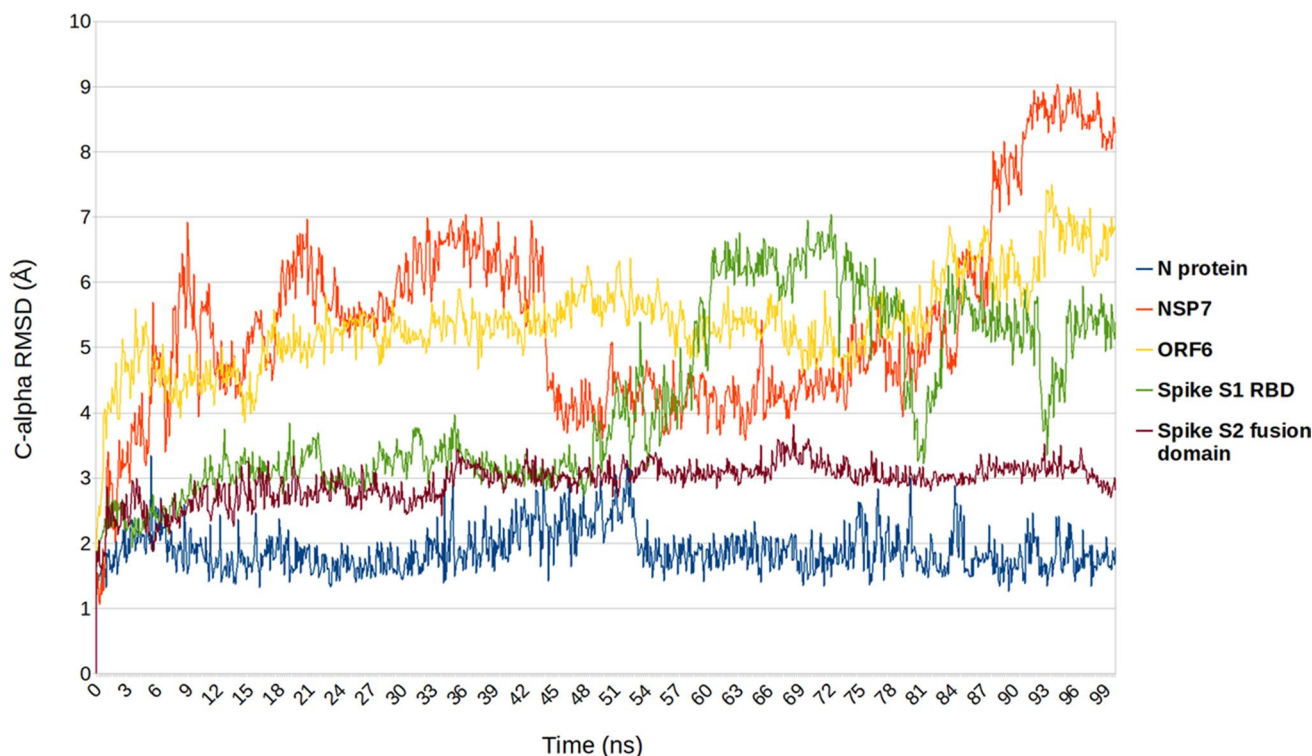

Fig. 2 C-alpha RMSD of different SARS-CoV-2 protein targets representing contact with Ivermectin in 100 ns simulation
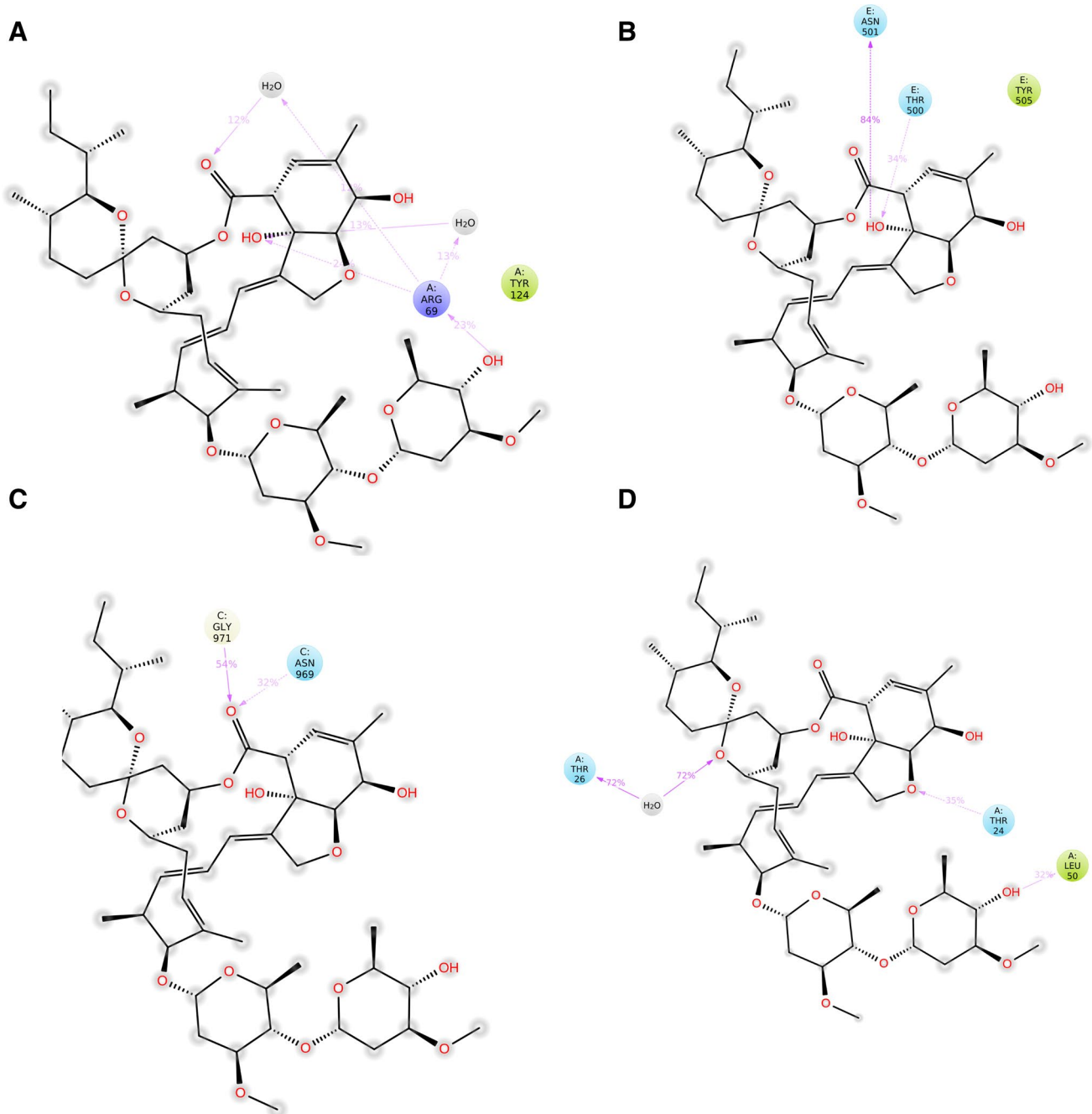
Fig. 3 2D contact with the protein targets of ivermectin with the interaction strength represented with adjacent active site residues (magenta continuous line: H-bond with Cα of protein; magenta dotted line: H-bond with side chain groups; green residue: hydrophobic; blue residue: polar residue, grey dots: solvent exposure) **a** N-protein contact with Ivermectin, **b** S1-RBD contact with Ivermectin, **c** S2 fusion peptide contact with Ivermectin, **d** CL protease contact with Ivermectin

to its exposed hydroxyl and ester groups that can highly assist as H-bond donor.

Discussion

From the evidence that is available and our artificial intelligence and molecular dynamics simulations based studies, ivermectin can be thought of as a potential drug for the treatment of COVID-19. Beneficial results have been observed with ivermectin in clinical studies. However, great diligence

Fig. 4 Ivermectin contact profile with SARS CoV-2 proteins **a** S1-RBD, **b** S2 fusion domain, **c** N protein NTD and **d** CL protease

Fig. 5 The 3D representation of ivermectin showing interaction points of ivermectin molecule in final bound trajectory frames with: **a** S1-RBD, **b** S2 fusion domain, **c** N protein NTD and **d** main protease (Yellow dotted line: H-bond, orange dotted line: indistinct weak interaction)

and regulatory review is required for testing of ivermectin in severe COVID-19 because of various reasons. As ivermectin targets the invertebrate's glutamate gated chloride channels, it can also cross-target mammalian GABA-gated chloride channels in the CNS. In normal conditions, this is prevented by BBB; however, in individuals having hyperinflammatory state, endothelial permeability at BBB may be enhanced, leading to drug leakage into the CNS and

neurotoxicity. Furthermore, anti-retroviral drugs used against SARS-CoV-2 like lopinavir/ritonavir and darunavir/cobicstat potently inhibit cytochrome P450 3A4 (ivermectin's main metabolic pathway) and if used concurrently with ivermectin can increase the systemic exposure to ivermectin. Ritonavir and cobicistat also inhibits P-glycoprotein efflux pump in BBB [34]. Moreover, well-controlled dose response study needs to be considered for carrying out a clinical trial of ivermectin. Schmith et al. carried out simulations with the help of available population pharmacokinetic model for predicting total and unbound plasma concentration–time profiles of ivermectin (200 µg/kg, 60 mg, and 120 mg) after administration of single and repeat fasted dose. According to their results, the IC50 value of ivermectin as reported by Caly et al. was much higher than the maximum plasma concentration achieved after administration of the above mentioned three doses of ivermectin when administered fasted. Hence, the chances of success of a trial that use the approved ivermectin dose (200 µg/kg) are less. They further suggested evaluation of use of combined therapy in vitro and ivermectin's inhaled treatment if feasible [35]. Furthermore, Momekove et al. also reported that according to pharmacokinetic data that is available from clinically relevant and excessive dosing studies SARS-CoV 2 in vitro inhibitory concentrations (5 µM/L) are not probable to be achievable in humans [36]. Next, ivermectin's cellular uptake by endothelial cells is limited, because it is highly bound (93%) to plasma proteins. Furthermore, ivermectin's total lung concentration reached only 100 ng/g (around 0.1 µM) in lung tissue in calves injected with 200 µg/kg, suggesting that accumulation of ivermectin would not be enough to accomplish the antiviral effect with conventional doses [37]. Jermain et al. developed a minimal physiologically-based pharmacokinetic model to simulate ivermectin's exposure to human lungs post oral doses (12, 30, and 120 mg). The simulated exposure of ivermectin to lungs achieved a concentration of 772 ng/mL, lower than the reported IC50 for ivermectin in vitro (1750 ng/mL) [38].

In molecular dynamics simulation studies, the interaction of ivermectin with multiple (four) viral targets with relatable specificity and nature of interaction suggest, i.e., which majorly involves rich H-bonds, can show inhibitory actions resembling the estimated outcomes from the MD simulations prototyped in physiological conditions. The binding coordinates of ivermectin observed were at the prime regions crucial for the activity of particular SARS-CoV proteins. The least structural deviation with the nucleocapsid protein N terminal domain (1.89 Å ± 0.33) and high interaction ratio points toward the suggestion that ivermectin exhibits relatively high affinity for N protein. The nucleocapsid shuttling has been proposed to be facilitated via human Importin α/β into the nuclear matrix [39, 40]. The reported binding of ivermectin to importin α/β and notably

low infection in ivermectin treated patients, might also possibly suggest that there is noticeable binding with the nucleocapsid cargo itself.

Conclusion

Hence, keeping in view the available evidence from clinical studies ivermectin can be a potential drug as it reduced mortality and improved symptoms of patients with COVID-19. Moreover, ivermectin in combination with doxycycline seems effective. However, more RCTs and dose response studies are required to justify its use. The molecular specificity of ivermectin seems to be quite assorted as there can be seen good binding profiles with spike S1 and S2 domains in addition to CL protease inhibition site. The marginally efficient binding to the Nucleocapsid (N) protein might point towards the idea that nucleocapsid activity gets affected after its trans-nuclear import. Hence, ivermectin might be involved in the inhibition of N protein (has a role in nuclear import) and as the exact mechanism is not known, we are describing the best possible target estimation for ivermectin. The findings incline the possibility of ivermectin to be a multi-targeted drug (host and virus-targeted) especially in the case of COVID-19.

Future recommendations

Ivermectin has been reported to show potent efficacy as an antiviral; however, its application is limited because of pharmacokinetic difficulties such as low solubility. These difficulties can be overcome by formulating liposomal ivermectin or other ivermectin formulations with improved properties. Furthermore, inhalation therapy of ivermectin can deliver high drug concentration to the lungs and airways to reduce the viral loads in such areas [41] or else it can be used in combination with other agents that differ in mechanism of action [38].

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