Mini-review and perspective

Ivermectin: a multifaceted drug of Nobel prize-honored distinction with indicated efficacy against a new global scourge, COVID-19

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Abstract

In 2015, the Nobel Committee for Physiology or Medicine, in its only award for treatments of infectious diseases since six decades prior, honored the discovery of ivermectin (IVM), a multifaceted drug deployed against some of the world's most devastating tropical diseases. Since March 2020, when IVM was first used against a new global scourge, COVID-19, more than 20 randomized clinical trials (RCTs) have tracked such inpatient and outpatient treatments. Six of seven meta-analyses of IVM treatment RCTs reporting in 2021 found notable reductions in COVID-19 fatalities, with a mean 31% relative risk of mortality vs. controls. The RCT using the highest IVM dose achieved a 92% reduction in mortality vs. controls (400 total subjects, $p<0.001$). During mass IVM treatments in Peru, excess deaths fell by a mean of 74% over 30 days in its ten states with the most extensive treatments. Reductions in deaths correlated with extent of IVM distributions in all 25 states with $p<0.002$. Sharp reductions in morbidity using IVM were also observed in two animal models, of SARS-CoV-2 and a related betacoronavirus. The indicated biological mechanism of IVM, competitive binding with SARS-CoV-2 spike protein, is likely non-epitope specific, possibly yielding full efficacy against emerging viral mutant strains.

Introduction

The 2015 Nobel prize for the discovery of ivermectin (IVM) and an antimalarial treatment was the Nobel committee's first award for treatment agents for infectious diseases since that of 1952 for streptomycin [1]. A macrocyclic lactone of multifaceted potency [2, 3], IVM as deployed worldwide since 1987 has made major inroads against two devastating tropical diseases, onchocerciasis and lymphatic filariasis [4]. During the year since IVM treatment of another global scourge, COVID-19, was first applied [5], results from more than 20 randomized clinical trials (RCTs) of IVM treatment of COVID-19 have been reported [2, 6, 7], with inpatient and outpatient treatments of COVID-19 conducted in 25 countries [2]. A likely biological mechanism has been indicated to be competitive binding with SARS-CoV-2 spike protein sites, as reviewed [8, 9].

Recently, Dr. Satoshi Omura, the Nobel co-laureate for the discovery of IVM, and colleagues conducted a comprehensive review of IVM clinical activity against COVID-19, concluding that the preponderance of the evidence demonstrated major reductions in mortality and morbidity [2]. Our review of that evidence, updated with consideration of several new studies, supports the same conclusion.

Animal studies for IVM treatment of SARS-CoV-2 and a closely related betacoronavirus

A framework for the examination of clinical IVM treatment results for COVID-19 is provided by related animal studies using IVM at low human-equivalent doses. In golden hamsters that were intranasally inoculated with SARS-CoV-2, causing symptomatic COVID-19 infections, concurrent dosing with IVM significantly reduced severity of clinical signs ($p<0.001$). While viral load was not reduced, these improvements included one-third the incidence of anosmia and sharp reductions in the Il-6/Il-10 ratio in lung tissue [10]. In another animal model, mice were infected with mouse hepatitis virus MHV-A59 [11], a betacoronavirus strain that does not express hemagglutinin esterase [12], like SARS-CoV-2, SARS-CoV, and MERS [8]. Whereas infected mice had severe histopathological liver damage, IVM-treated mice had half the hepatic viral load and minimal liver damage not significantly different than that observed in uninfected controls.

RCTs for IVM treatment and prevention of COVID-19

More than 20 RCTs for IVM treatment of COVID-19 have been conducted to date, as cited above. A search of Google Scholar for meta-analyses of IVM treatment studies of COVID-19 that appeared in 2021 [13] yielded seven such studies that drew conclusions from RCTs only [6, 14-19]. The relative risk (RR) of mortality with IVM treatment vs. controls as calculated in four of these meta-analyses using Cochrane analysis methodology ranged from 0.25 to 0.37, with a mean of 0.31 [6, 14, 15, 19]. The three other meta-analyses reported odds ratios of 0.16, 0.21 and 0.33, with a mean of 0.23 [16-18]. Six of these seven meta-analyses concluded that there was a significant [6, 14-16] or possible [17, 18] indication of efficacy of IVM in reducing COVID-19 mortality. One found no evidence of IVM efficacy in its first version [20], reporting an RR of 1.11 for IVM treatment vs. controls, and stuck with that finding even after changing this RR value to 0.37 and correcting switched treatment and control deaths it had misreported for one study [21] in a revised version [19]. Among the most recent and comprehensive of these seven meta-analyses reported a pooled total of 31 deaths among 1,101 subjects in IVM treatment groups and 91 deaths among 1,064 controls from 11 RCTs, amounting to a 67% reduction in mortality, with a statistical significance for overall effect of $p=0.005$ [16]. The RCT that used the largest dose of IVM, 400 μg/kg on each of days 1-4 [22], had 2 vs. 24 deaths in the treatment vs. control groups (n=200 each), a 92% reduction in COVID-19 mortality ($p<0.001$).

An objection that had been raised earlier in 2021 to the preponderance of clinical evidence for efficacy of IVM treatment of COVID-19 as summarized above was that none of these RCTs had been published in mainstream peer-reviewed scientific journals [23]. Closing that gap, however, was the publication in 2021 in journals from major scientific publishers of five such RCTs for COVID-19 treatment [24-28], each showing multiple clinical benefits for IVM vs. controls, most of these to statistical significance at $p<0.002$. Also published in 2021 were three other RCTs for IVM treatment of COVID-19: one that reported briefer hospital stays for IVM treatment short of statistical significance ($p=0.08$) [29], another that compared IVM with two other drug treatment groups but not a placebo group and found no benefit

[30], and an additional study conducted in Cali, Columbia with mix-ups between treatment and placebo doses as described below.

Another objection that has been raised to the RCT evidence supporting IVM efficacy was that study populations were too small [31]. Yet it is well known in clinical trial design that highly effective drugs will establish statistically significant results with smaller sample sizes, with larger study populations required for minimally effective drugs [32]. For example, as noted above, the highest dose IVM treatment study for COVID-19 that tracked mortality had 2 vs. 24 deaths in treatment vs. control arms of 200 subjects each [22], with a z test p-value of 0.0006 [33]. But for a drug with a more modest RR of 75%, for example, the treatment and control arms would need more than 3,800 subjects each to yield the same statistical significance [33]. Although large study populations are useful to screen for adverse effects (AEs) of new drugs, IVM has been used safely in 3.7 billion doses worldwide since 1987 [2, 3] and is well tolerated even at much greater than the standard single dose of 200 μg/kg [34, 35]. It has been used in RCTs for COVID-19 treatment at cumulative doses of 1,500 μg/kg [36], 1,600 μg/kg [22] and 3,000 μg/kg[37] over 4 or 5 days with only small percentages of mild or transient adverse effects.

Among these RCTs that established safety for high-dose IVM treatment of COVID-19 was one conducted in Cali, Columbia with generally mild COVID-19 cases, median age 37, having only one death in the control group [36]. The study found no statistically significant symptom improvements with IVM treatment, yet reported a striking anomaly: AEs distinctive for its high IVM dose, described in the study protocol as “security parameters” for its IVM use, occurred at almost identical rates in its IVM and placebo arms. These included transient incidences of blurred vision (11.3%, 11.6%) and dizziness (35.6%, 34.3%). These indications of IVM use in controls occurred as over-the-counter sales of IVM surged in the study region during the study period (Supplementary Table 1). Further questions as to the study’s treatment/control boundaries were raised by the mistaken substitution of IVM for placebo for 38 patients, discovered by the lead pharmacist a month after the fact (study, p. 3; study protocol supplement, p. 43). In addition, blinding was breached by the use of dextrose-saline solution as the placebo for 64

control patients (IVM tastes distinctively bitter), while the composition of the replacement placebo solution was not specified [38].

Supporting the findings of IVM efficacy in COVID-19 treatment as summarized above were indications of activity against SARS-CoV-2 in prevention studies. Three RCTs evaluated the prophylactic effect of IVM administered to cohorts of 100 [22], 117 [39] and 203 [40] subjects exposed to COVID-19 patients. These studies, all using IVM in doses of at least 150 μg/kg per week, reported statistically significant reductions in COVID-19 incidences, with respective RRs of 20%, 26% and 13% as compared with controls, and greater reductions in incidences of moderate and severe cases. Another RCT for COVID-19 prevention administered just one dose of IVM at 12 mg (about 150 μg/kg) to 617 subjects on day one of a 42-day observation period, while three other preventative regimens were each administered daily over that period [41]. IVM at that single low dose yielded the best results of these four regimens, with highly statistically significant reductions of close to 50% in both symptomatic COVID-19 and acute respiratory symptoms vs. controls.

14-fold reductions in excess deaths with IVM use in Peru, then 13-fold increase after IVM restricted

The clinical experience of IVM treatments of COVID-19 in 25 countries extends far beyond the RCT results summarized, yet incomplete tracking and lack of control data exclude most of this for evaluation. The record of nationally authorized such treatments in Peru provides a notable exception [42]. In ten states of Peru, mass IVM treatments of COVID-19 were conducted through a broadside, army-led effort, *Mega-Operación Tayta* (*MOT*), that began on different dates in each state. In these *MOT* states, excess deaths dropped sharply over 30 days from peak deaths by a mean of 74%, in close time conjunction with *MOT* start date (Figure 1B). In 14 states of Peru having locally administered IVM distributions, the mean reduction in excess deaths over 30 days from peak deaths was 53%, while in Lima, which had minimal IVM distributions during the first wave of the pandemic due to restrictive government policies there, the corresponding 30-day decrease in excess deaths was 25%.

Reductions in excess deaths by state (absolute values) correlated with extent of IVM distribution (maximal-*MOT* states, moderate-local distributions, and minimal-Lima) with Kendall τ_b = 0.524, $p<0.002$, as shown in Figure 1C. Nationwide, excess deaths decreased 14-fold over four months through December 1, 2020. After a restrictive IVM treatment policy was enacted under a new Peruvian president who took office on November 17, however, deaths increased 13-fold over the two months following December 1, through February 1, 2021 (Figure 1A). Potential confounding factors, including lockdowns and herd immunity, were ruled out using Google community mobility data, seropositivity rates, population densities and geographic distributions of SARS-CoV-2 genetic variations and by restricting all analysis except that for Figure 1A to ages $\geq$ 60. Excess deaths was used in all analyses rather than COVID-19 case fatalities as gross underreporting of pandemic deaths by case fatalities was known to the Peruvian ministry of health since July 2020 [43]. This disparity has been consistently manifested in the national health database figures for COVID-19 case fatalities vs. all natural-cause deaths since that date [42].

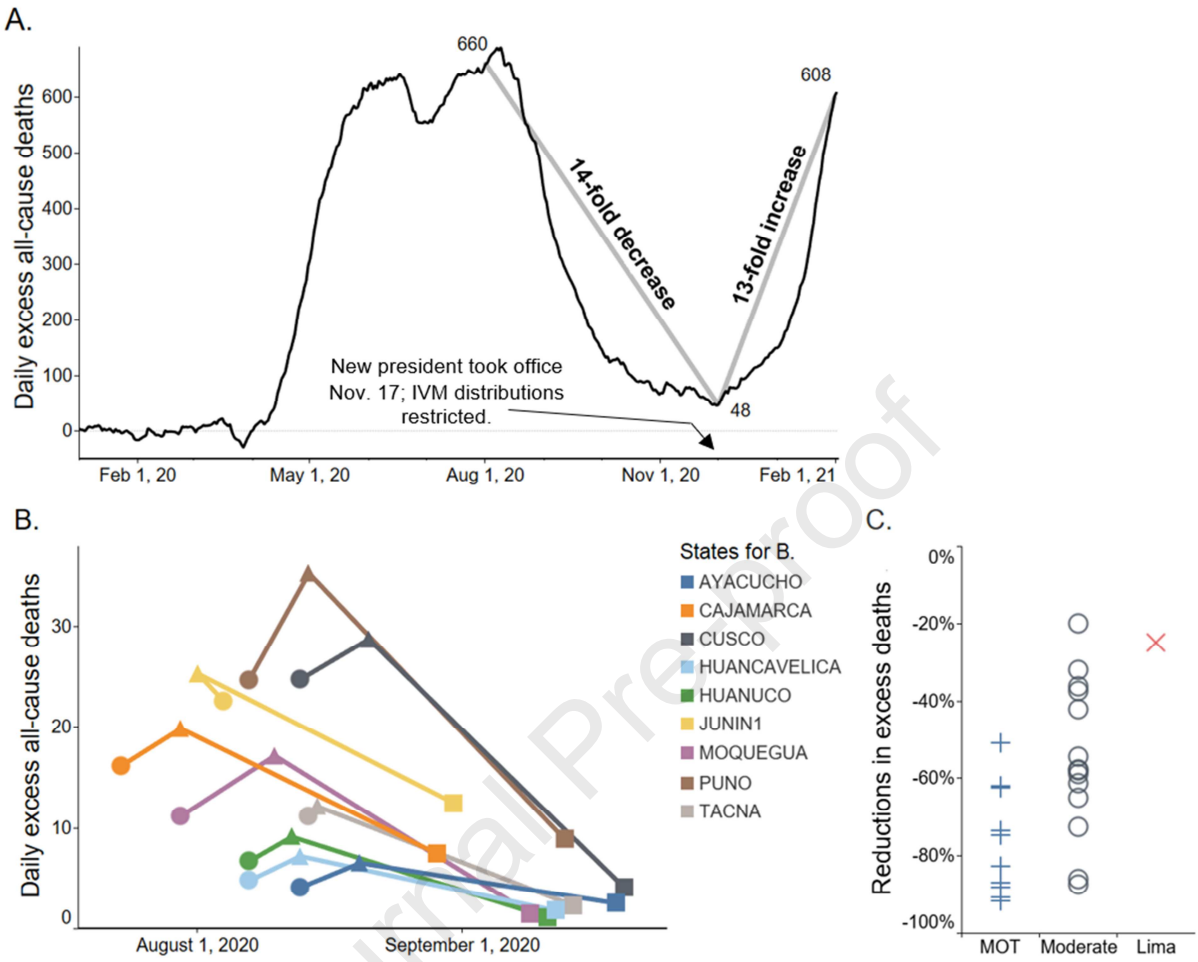

Figure 1. A) Excess all-cause deaths (all ages), national population of Peru. These decreased 14-fold August 1 through December 1, 2020; then, after IVM use was restricted, increased 13-fold through February 1. For A and B, y values are 7-day moving averages; for B and C, ages ≥ 60. Data are from Peru's National Death Information System (SINADEF). B) Drops in excess deaths for all states of operation *MOT*, an army-led program of mass IVM distributions, but Pasco, which had them on 3 dates. ● *MOT* start date; ▲ peak deaths; ■ day of peak deaths + 30 days. Junin distributed IVM through local channels 13 days before *MOT* start. C) Reductions in excess deaths at +30 days after peak deaths for the 25 states by extent of IVM distributions: maximal-*MOT* (+), mean -74%; moderate-local distributions ( ), mean -53%; and minimal-Lima (x), -25%. The absolute value of these reductions by state correlated with extent of IVM distributions with Kendall τ_b = 0.524, p<0.002 (Spearman rho=0.619, p<0.001). All these data are from publicly accessible Peruvian national databases, with associated frozen datasets available from the Dryad data repository [42].

IVM-based combination treatments and other research in progress

Combination treatments using IVM and adjuncts have shown indications of efficacy against COVID-19 in RCTs conducted to date [24, 44]. Results using IVM, doxycycline and zinc to treat serious and critical cases having spO2 ≤ 90 prior to treatment, with spO2 changes tracked 24 hours after treatment, will be reported by TJB with Sabine Hazan, MD. Pronounced improvements of serious COVID-19 symptoms within 1-2 days after IVM administration have been observed in several patients treated by the lead author (ADS), and studies to objectively track such short-term clinical benefits of IVM for COVID-19 are underway. Information on other combination treatments using IVM with agents such as fluvoxamine, for which clinical studies also indicate significant benefits [45], is provided by the USA-based FLCCC alliance (https://covid19criticalcare.com).

The curative potential of combination therapy was demonstrated in a medical breakthrough of three decades prior for another disease, peptic ulcers, for which the discovery of its underlying bacterial cause, *Helicobacter pylori*, was honored with the Nobel Prize for Medicine, in 2005. In 1990, Dr. Thomas J. Borody published the original clinical trial of a combination treatment for *H. pylori*, achieving a 96% cure rate for a triple therapy consisting of three repurposed drugs, bismuth subcitrate and two antibiotics [46]. Between 1990 and 2015, an estimated 18,665 deaths were prevented by the timely application of this triple therapy for peptic ulcer disease in Australia [47]. After expiration of the patents for two palliative drugs for this condition, Tagamet and Zantac [48], which had each earned billions of dollars, triple therapy became the standard of care for peptic ulcers in the rest of the world by the late 1990s.

Conclusion

We believe that the evidence to date supports the worldwide extension of IVM treatments for COVID-19, complementary to immunizations. The indicated biological mechanism of IVM,

competitive binding with SARS-CoV-2 spike protein, is likely non-epitope specific, as reviewed [8], possibly yielding full efficacy against emerging viral mutant strains. IVM has been safely used in 3.7 billion doses since 1987, well tolerated even at much greater than standard doses [34, 35] and used without serious AEs in the three high-dose COVID-19 treatment studies noted above [34, 36, 37]. In the current international emergency of COVID-19, with mutant viral strains, vaccination refusals and potentially waning immunities over months presenting new challenges, IVM can be an effective component of the mix of therapeutics deployed against this pandemic.

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Ethical approval and consent to participate

This is a review and ethical approval was not required.

Conflict of interest. TJB is a principal in Topelia Therapeutics (Ventura, California), which seeks to commercialize cost-effective treatments for COVID-19, including IVM. All other authors report no conflicts of interest.

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Short Communication

A five-day course of ivermectin for the treatment of COVID-19 may reduce the duration of illness

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ABSTRACT

Ivermectin, a US Food and Drug Administration-approved anti-parasitic agent, was found to inhibit severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replication in vitro. A randomized, double-blind, placebo-controlled trial was conducted to determine the rapidity of viral clearance and safety of ivermectin among adult SARS-CoV-2 patients. The trial included 72 hospitalized patients in Dhaka, Bangladesh, who were assigned to one of three groups: oral ivermectin alone (12 mg once daily for 5 days), oral ivermectin in combination with doxycycline (12 mg ivermectin single dose and 200 mg doxycycline on day 1, followed by 100 mg every 12 h for the next 4 days), and a placebo control group. Clinical symptoms of fever, cough, and sore throat were comparable among the three groups. Virological clearance was earlier in the 5-day ivermectin treatment arm when compared to the placebo group (9.7 days vs 12.7 days; $p = 0.02$), but this was not the case for the ivermectin + doxycycline arm (11.5 days; $p = 0.27$). There were no severe adverse drug events recorded in the study. A 5-day course of ivermectin was found to be safe and effective in treating adult patients with mild COVID-19. Larger trials will be needed to confirm these preliminary findings.

Introduction

Coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a global pandemic of the highest priority (Johns Hopkins University of Medicine, 2020). Eighty-one percent of cases are categorized as mild, for whom symptomatic management at home and monitoring of clinical deterioration is recommended (Centers for Disease Control and Prevention, 2020). Despite providing symptomatic management, a therapeutic drug that would limit the course of infection is greatly needed.

Ivermectin, a popular anti-parasitic drug, acts on SARS-CoV-2 by preventing viral proteins from entering the host cell nucleus (Caly et al., 2020). Recent virtual drug screening identified doxycycline as a potential inhibitor of SARS-CoV-2 papain-like protease (Wu et al., 2020). An observational study in which patients were treated with a single-dose of ivermectin and multiple doses of doxycycline for the treatment of COVID-19 yielded considerable improvements in symptoms and the viral response (Alam et al., 2020). A recent retrospective study found that hospitalized patients given ivermectin with other treatments (e.g., azithromycin and hydroxychloroquine) had a lower mortality than those who did not receive ivermectin (Rajter et al., 2020). Further studies are needed to verify these findings. This need is further underscored by the observation that SARS-CoV-2 multiplies rapidly in the respiratory tract and that evidence from animal models shows three-fold higher levels of ivermectin in pulmonary tissue than in the plasma at 1 week after oral dosing (Chiu and Lu, 1989; Lespine et al., 2005). This pilot study was performed to evaluate the rapidity of viral clearance and safety of a 5-day course of ivermectin or a single-dose of ivermectin + a 5-day course of doxycycline in the treatment of mild COVID-19 in adults.

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Methods

A randomized, double-blind, placebo-controlled trial was conducted to determine the rapidity of viral clearance and safety of ivermectin among adult SARS-CoV-2 patients. The trial included 72 hospitalized patients in Dhaka, Bangladesh, who were assigned to one of three groups: oral ivermectin alone (12 mg once daily for 5 days), oral ivermectin in combination with doxycycline (12 mg ivermectin single dose and 200 mg doxycycline on day 1, followed by 100 mg every 12 h for the next 4 days), and a placebo control group. Inclusion criteria were age 18–65 years; admitted to hospital within the last 7 days; presence of a fever (≥37.5 °C), cough, and/or sore throat; diagnosed positive for SARS-CoV-2 by real-time reverse transcription PCR (rRT-PCR). Patients were excluded if they were allergic to ivermectin or doxycycline, or if there was the potential for a drug–drug interaction with ivermectin or doxycycline; had chronic illnesses (e.g., ischemic heart disease, heart failure, documented cardiomyopathy, chronic kidney disease, chronic liver disease); had received ivermectin and/or doxycycline in the last 7 days; were pregnant or lactating; or had participated in any other clinical trial within the last month.

Patients underwent a physical examination for COVID-19-related symptoms and their vital signs were recorded (e.g., temperature, blood pressure, pulse rate, oxygen saturation, and respiratory rate). Nasopharyngeal swabs were obtained to confirm the presence of SARS-CoV-2 by rRT-PCR on the day of enrolment, and then on days 3, 7, and 14. After day 14, patients were followed-up weekly until found to be test-negative.

Venous blood was collected for blood parameters on enrolment and on day 4 (complete blood count, creatinine, alanine aminotransferase, Random Blood Sugar). A chest X-ray and ECG were assessed on enrolment and on day 3. Blood biomarkers were measured on enrolment and on day 7 (C-reactive protein (CRP), ferritin, lactose dehydrogenase (LDH), and procalcitonin). RNA was tested for SARS-CoV-2 by rRT-PCR targeting ORF1ab- and N-gene specific primers and probes following the protocol of the Chinese Center for Disease Control and Prevention; subjected to amplification (iTaq Universal Probes One-Step Kit; Bio-Rad Laboratories, Inc., Hercules, CA, USA) in a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules CA, USA). A positive case had a cycle threshold (Ct) value of <40. Other information collected included demographic data and details of any co-morbidity, medication use, and previous hospitalization as part of the medical history. Data were entered into SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA).

The primary endpoints were the time required for virological clearance (a negative rRT-PCR result on nasopharyngeal swab), and remission of fever (≥37.5 °C) and cough within 7 days. Secondary outcomes included failure to maintain an SpO_2 >93% despite oxygenation and days on oxygen support, the duration of hospitalization, and all-cause mortality. Drug safety outcomes recorded were adverse events that occurred during treatment and post treatment, and the discontinuation of the study drug during the trial.

Results

Study descriptors

A total of 72 out of 113 patients who consented were enrolled in the trial; 24 patients were included per study arm. One patient from each of the ivermectin + doxycycline and placebo groups and two patients in the 5-day ivermectin group withdrew their consent during the study due to family obligations and unwillingness to be tested further. The pre-treatment characteristics (demographics, clinical history, co-morbidity, and laboratory values) were comparable among the three treatment groups. The mean age was 42 years and 54% were female. The duration of illness before assessment was an average of 3.83 days.

The mean duration of hospitalization after treatment was 9.7 days (95% confidence interval (CI) 8.1–11.0 days) in the placebo group, 10.1 days (95% CI 8.5–11.8 days) in the ivermectin + doxycycline group, and 9.6 days (95% CI 7.7–11.7 days) in the ivermectin alone group ($p = 0.93$). None of the patients enrolled required oxygen or had serious adverse drug events recorded. The mean values of the blood biomarkers (CRP, LDH, procalcitonin, and ferritin) dropped from baseline to day 7 in all three groups and these changes were significant for CRP ($p = 0.02$) and LDH ($p = 0.01$) in the 5-day ivermectin arm and for LDH in the placebo group ($p = 0.01$).

At enrolment, 82.6% (19/23) of patients in the placebo group, 73.9% (17/23) in the ivermectin + doxycycline group, and 77.3% (17/22) in the 5-day ivermectin group were recorded as having a fever, among whom 84.2% (16/19), 94.1% (16/17), and 100% (17/17), respectively, were afebrile on day 7. Similarly, 65.2% (15/23) in the placebo group, 82.6% (19/23) in the ivermectin + doxycycline group, and 81.8% (18/22) in the 5-day ivermectin group had a cough on enrolment. On day 7, this dropped to 40% (9/15), 63.2% (7/19), and 61.1% (7/18), respectively, for cough. Sore throat was present at enrolment in 17.4% (4/23), 13% (3/23), and 18.2% (4/22) of patients in the placebo group, ivermectin + doxycycline group, and 5-day ivermectin group, respectively, and on day 7, the sore throat had subsided in 75% (3/4), 33.3% (1/3), and 75% (3/4) of patients, respectively. Of note, these changes were not statistically significant for fever ($p = 0.35$ and $p = 0.09$), cough ($p = 0.18$ and $p = 0.23$), or sore throat ($p = 0.35$ and $p = 0.09$) in the ivermectin + doxycycline and the 5-day ivermectin groups when compared with placebo.

Viral clearance

The mean duration to viral clearance was 9.7 days (95% CI 7.8–11.8 days) for the 5-day ivermectin arm ($p = 0.02$), 11.5 days (95% CI 9.8–13.2 days) for the ivermectin + doxycycline ($p = 0.27$) arm, and 12.7 days (95% CI 11.3–14.2 days) for the placebo group. Kaplan–Meier survival analysis revealed that the proportion of patients at risk of SARS-CoV-2 was significantly reduced in the 5-day ivermectin group (Figure 1, below). Virological clearance in the 5-day ivermectin group was significantly earlier compared to the placebo group on days 7 and 14 (hazard ratio (HR) 4.1, 95% CI 1.1–14.7 ($p = 0.03$) and HR 2.7, 95% CI 1.2–6.0 ($p = 0.02$)). The trend was similar for the ivermectin + doxycycline group on days 7 and 14, but this was not statistically significant (HR 2.3, 95% CI 0.6–9.0 ($p = 0.22$) and HR 1.7, 95% CI 0.8–4.0 ($p = 0.19$)).

Discussion

The drugs ivermectin and doxycycline are commonly used in the developing world and have been found to be safe and effective in treating both parasitic and bacterial infections. The drugs are affordable (the full 5-day cost ranges from US$ 0.60 to US$ 1.80 for 5-day ivermectin) and readily available in Bangladesh, and thus are a highly attractive alternative for treating COVID-19 patients. The aim of this study was to investigate the role of ivermectin alone or in combination with doxycycline in the treatment of adult COVID-19 patients presenting with mild symptoms. It was hoped that treatment early in the course of infection would decrease the viral load, shorten the duration of illness, and halt transmission.

A 5-day course of ivermectin resulted in an earlier clearance of the virus compared to placebo ($p = 0.005$), thus indicating that early intervention with this agent may limit viral replication within the host. In the 5-day ivermectin group, there was a significant drop in CRP and LDH by day 7, which are indicators of disease severity. It is noteworthy that the viral nucleic acid Ct value (indicator of viral load) dropped significantly compared to the placebo group on day 7

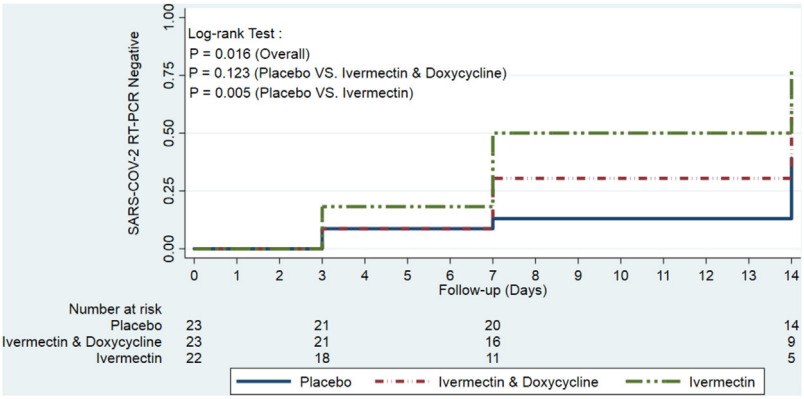

Figure 1. Cumulative viral recovery estimates in the overall study population.

and day 14. In the absence of co-morbidity, a 5-day course of ivermectin treatment showed faster SARS-CoV-2 virus clearance compared to the placebo arm (9 vs 13 days; $p = 0.02$).

Although the study sample was too small ($n = 72$) to draw any solid conclusions, the results provide evidence of the potential benefit of early intervention with the drug ivermectin for the treatment of adult patients diagnosed with mild COVID-19. First, early intervention promoted faster viral clearance during disease onset, which might have prevented significant immune system involvement and hastened the recovery. Secondly, early intervention reduced the viral load faster, thus may help block disease transmission in the general population. A larger randomized controlled clinical trial of ivermectin treatment appears to be warranted to validate these important findings.

Funding source

This work was supported by Beximco Pharmaceutical Limited, Bangladesh.

Ethical review

The trial was approved by the Institutional Review Board (Research Review Committee and Ethical Review Committee) of icddr,b and subsequently by the National Ethics Review Committee of Bangladesh Medical Research Council and Clinical Trial Advisory Committee of the Directorate General of Drug Administration, Government of Bangladesh. Written informed consent was obtained from all patients.

Declaration of interests

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

Conflicts of interest

The authors have no conflicts of interest to declare.

Author contributions

JDC, AGR, WAK, KZ, JS, conceived and designed the study. RY, MAH, AK, SA, MMK, CSP, MSH, MR, and ABA made substantial contributions in reviewing the design of the study and acquiring the data. SA, MMK, MSH, and ABA coordinated sample collection and oversaw data collection. MR and MKS conducted and analysed the laboratory results. MSH analysed the data and WAK, SA, MMK, CSP and MSH interpreted the data. WAK and SA conducted the literature review and drafted the manuscript. MMK, MSH, and ABA contributed by revising the manuscript critically for important intellectual content. JDC, AGR, WAK, KZ, JS, MSF, MR, RY, MAH, and AK critically reviewed the manuscript. All authors contributed to final approval of the version to be submitted.

Author agreement

All authors have seen and approved the final version of the manuscript being submitted. The article is the authors' original work, has not received prior publication, and is not under consideration for publication elsewhere.

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OPINION | COMMENTARY

Why Is the FDA Attacking a Safe, Effective Drug?

Ivermectin is a promising Covid treatment and prophylaxis, but the agency is denigrating it.

By David R. Henderson and Charles L. Hooper
July 28, 2021 12:34 pm ET

Ivermectin in Buenos Aires, Jan. 26.
PHOTO: ROBERTO ALMEIDA AVELEDO/ZUMA PRESS

The Food and Drug Administration claims to follow the science. So why is it attacking ivermectin, a medication it certified in 1996?

Earlier this year the agency put out a special warning that "you should not use ivermectin to treat or prevent COVID-19." The FDA's statement included words and phrases such as "serious harm," "hospitalized," "dangerous," "very dangerous," "seizures," "coma and even death" and "highly toxic." Any reader would think the FDA was warning against poison pills. In fact, the drug is FDA-approved as a safe and effective antiparasitic.

Ivermectin was developed and marketed by Merck & Co. while one of us (Mr. Hooper) worked there years ago. William C. Campbell and Satoshi Omura won the 2015 Nobel Prize for Physiology or Medicine for discovering and developing avermectin, which Mr. Campbell and associates modified to create ivermectin.

Ivermectin is on the World Health Organization's List of Essential Medicines. Merck has donated four billion doses to prevent river blindness and other diseases in Africa and other places where parasites are common. A group of 10 doctors who call themselves the Front Line Covid-19 Critical Care Alliance have said ivermectin is "one of the safest, low-cost, and widely available drugs in the history of medicine."

Ivermectin fights 21 viruses, including SARS-CoV-2, the cause of Covid-19. A single dose reduced the viral load of SARS-CoV-2 in cells by 99.8% in 24 hours and 99.98% in 48 hours, according to a June 2020 study published in the journal Antiviral Research.

Some 70 clinical trials are evaluating the use of ivermectin for treating Covid-19. The statistically significant evidence suggests that it is safe and works for both treating and preventing the disease.

In 115 patients with Covid-19 who received a single dose of ivermectin, none developed pneumonia or cardiovascular complications, while 11.4% of those in the control group did. Fewer ivermectin patients developed respiratory distress (2.6% vs. 15.8%); fewer required oxygen (9.6% vs. 45.9%); fewer required antibiotics (15.7% vs. 60.2%); and fewer entered intensive care (0.1% vs. 8.3%). Ivermectin-treated patients tested negative faster, in four days instead of 15, and stayed in the hospital nine days on average instead of 15. Ivermectin patients experienced 13.3% mortality compared with 24.5% in the control group.

Moreover, the drug can help prevent Covid-19. One 2020 article in Biochemical and Biophysical Research Communications looked at what happened after the drug was given to family members of confirmed Covid-19 patients. Less than 8% became infected, versus 58.4% of those untreated.

Despite the FDA's claims, ivermectin is safe at approved doses. Out of four billion doses administered since 1998, there have been only 28 cases of serious neurological adverse events, according to an article published this year in the American Journal of Therapeutics. The same study found that ivermectin has been used safely in pregnant women, children and infants.

If the FDA were driven by science and evidence, it would give an emergency-use authorization for ivermectin for Covid-19. Instead, the FDA asserts without evidence that ivermectin is dangerous.

At the bottom of the FDA's warning against ivermectin is this statement: "Meanwhile, effective ways to limit the spread of COVID-19 continue to be to wear your mask, stay at least 6 feet from others who don't live with you, wash hands frequently, and avoid crowds." Is this based on the kinds of double-blind studies that the FDA requires for drug approvals? No.

Mr. Henderson, a research fellow with the Hoover Institution at Stanford University, was senior health economist with President Reagan's Council of Economic Advisers. Mr. Hooper is president of Objective Insights, a firm that consults with pharmaceutical clients.

Correction
This article has been edited to remove a reference to a study of 200 healthcare workers by Ahmed Elgazzar of Benha University in Egypt. Messrs. Henderson and Hooper relied on a summary of studies published in the American Journal of Therapeutics. They learned after publication that this study has been retracted because of charges of data manipulation.

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Letter to the editor

Ivermectin: potential candidate for the treatment of Covid 19

Dear Editor:

Ivermectin, a well-known anti-helmintic agent from the late-1970s, causes stimulation of gamma amino butyric acid (GABA)-gated-Cl^- channels, leading to hyperpolarization, and resulting in paralysis of the infesting organism. Another mechanism that has been postulated for the same effect is the immunomodulation of host response. This is attained by the activation of neutrophils, increase in the levels of C-reactive protein and interleukin-6.[1] In recent times, the antiviral function of ivermectin has been discovered, which appears to be intriguing. Already its effectiveness against certain flavivirus (dengue fever, Japanese encephalitis and tick-borne encephalitis virus) and chikungunya virus has been demonstrated *in vitro*.[2,3] Since then the same activity has been assessed in numerous other viral infections. Off lately its potency has been recognized in eliminating coronavirus *in vitro*. The exact mechanism to which this effect can be attributed to is yet to be validated, but the speculated method is inhibition of importin α/β1 mediated transport of viral proteins in and out of the nucleus.[4] Importins, a type of karyopherins, exemplify a major class of soluble transport receptors which are involved in nucleo-cytoplasmic transit of various substrates (Fig. 1).[5] The speculated inhibitory action of ivermectin on importin α/β mediated transport system, Based on this conjecture, the role of ivermectin in eliminating Covid-19 can be assumed.

Until now, in only single *in vitro* study, the efficacy of ivermectin against coronavirus has been demonstrated. Caly et al. tested for the viral RNA levels in both supernatant and cell pellets of the Vero/hSLAM cells which were infected with SARS-CoV-2 (isolate Australia/VIC01/2020), and were then treated with 5 μM ivermectin two hours later. After 24 h, they observed a decline of about 93% and 98% in viral RNA levels and cell-associated viral RNA, respectively. Later at 48 h, they detected further reduction (~5000 fold) in the viral RNA load only. To ascertain this finding, the infected cells were treated with serial dilutions of ivermectin, and were then tested for viral RNA load by RT-PCR. With this research, the investigators could comment about the inhibitory concentration 50 (IC50) which was estimated to be ~2 μM, and also that no toxicities

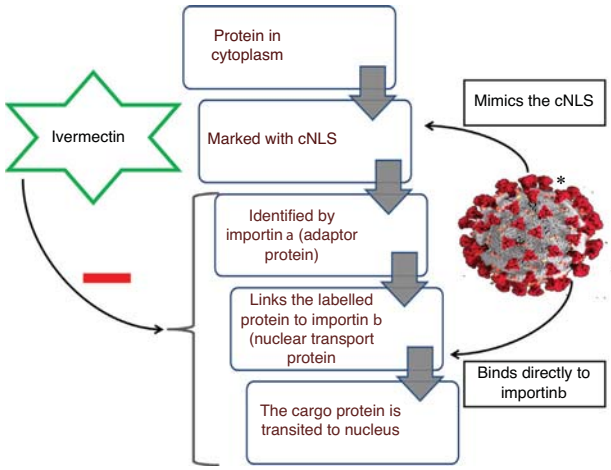

Fig. 1 – Mechanism of ivermectin induced inhibition of importin α/β mediated coronavirus proteins transport. cNLS : classical Nuclear Localization Signal. *Image courtesy: CDC/Alissa Eckert, MS; Dan Higgins, MAMSA.

were noticed for the various concentrations at which ivermectin was tested.[6] Based on the efficacy evidenced in *in vitro* study, various clinical studies have been planned and started, though none of them have yet been completed (Table 1).

The *in vitro* potency of ivermectin against Covid-19 virus is a testimony that this drug can be utilized to manage those patients who have been infected with SARS-CoV-2. Since the conditions in which the virus replicates and infects the cells *in vivo* and *in vitro* differs, a decisive comment about how ivermectin may prove to be beneficial to the patients cannot be constructed yet. Similarly, any disparity in the pharmacokinetic properties of this drug and the unidentified drug interactions which may occur under such conditions are yet to be recognized and remarked on. Nevertheless if compared with the other pharmacotherapeutic options for the management of Covid-19 infection, ivermectin may prove to have leverage over them. In addition to a different mechanism of action, there are other facets as well in which this drug may have an upper hand. For instance, the adverse

Table 1 – Salient features of ongoing clinical trials of ivermectin for COVID-19.

S. No	Intervention	Phase	No. of Participants	Primary End Point(s)	Clinical Trial Identifier
1	Ivermectin 0.2 mg /kg (single dose at once = 2 tablets of 6 mg/weekly) Hydroxychloroquine 400 mg/daily Azithromycin capsules 500 mg daily Placebo	I	50	Number of patients cured assessed by Nasopharyngeal swab, oropharyngeal swab, and blood aspiration for covid19 (PCR) in addition to chest x-ray in 14 days	NCT04343092
2	Ivermectin 600 μg / kg once daily plus standard care. Control: Standard Care	II	45	Number of patients in whom the SARS-CoV-2 viral load decreases after ivermectin treatment in 1–5 days	NCT04381884
3	Bicalutamide 150 mg by mouth daily for 7 days Ivermectin 600 μg/kg (up to a maximum dose of 60 mg) by mouth daily for 3 days	II	60	Number of participants who have clinical improvement at day 7 after randomization	NCT04374279
4	Hydroxychloroquine: Days 1–14: 3 tabs (600 mg total daily dose) Azithromycin: Day 1: 2 tabs (500 mg total daily dose) Days 2–5: 1 tab (250 mg total daily dose) Ivermectin: Days 1–2: Weight < 75 kg: 4 tabs (12 mg total daily dose) Days 1–2: Weight > 75 kg: 5 tabs (15 mg total daily dose) Camostat Mesilate Days 1–14: 2 tab TID after a meal (600 mg total daily dose)	II	240	Proportion of patients experiencing clinical deterioration in 14 days	NCT04374019
5	Ivermectin 200 μg/kg once orally plus Nitazoxanide 500 mg twice daily orally with meal for 6 days Control: Standard Care	II/III	100	Number of Patients with COVID-19-negative PCR in 10 days	NCT04360356
6[a]	Chloroquine Chloroquine with Nitazoxanide Chloroquine with ivermectin	II III	60	Number of patients with virological cure in six months	NCT04351347
7[a]	Chloroquine Favipiravir Nitazoxanide Ivermectin Niclosamide Other drugs (oselatamivir or combination of any of above treatment)	II / III	120	Number of patients with decreased viral load in six months	NCT04345419
8[a]	Nitazoxanide Ivermectin Chloroquine Azithromycin	III	80	Number of patients with virological cure in six months	NCT04382846
9	Ivermectin 200–400 μg per kg body weight Control: Standard Care	N/A	50	Test for virus at 1, 3 & 5 days from beginning of trial drug started for the patient in the hospital in 03 months	NCT04373824

All the details mentioned, have been obtained from https://clinicaltrials.gov/.

[a] Dose of the drugs not available.

effects associated with hydroxychloroquine (irreversible retinal damage, prolong QT interval, myopathy, neuropathy) or with lopinavir + ritonavir combination (hypertriglyceridemia, hypercholesterolemia) are not seen in patients who are on ivermectin. Furthermore, the treatment regimen with ivermectin may turn out to be more cost-effective. The therapeutic regimen with hydroxychloroquine and azithromycin combination comes out to be ~5–6 times more expensive than the

one with ivermectin. The same can be commented about the patent antivirals which are priced at exorbitant rates. Another worthwhile issue to be addressed is the over-utilization of hydroxychloroquine in managing the Covid-19 patients, may create an apparent shortage of this drug which is a standard treatment for patients with auto-immune diseases.

Taking into account these lacunae and merits, it becomes imperative that clinical trials with ivermectin be conducted in patients of Covid-19, to comprehend whether this drug can provide beneficial effect to those patients who have already developed complications due to this infection.

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Conflict of interest

The authors declare no conflicts of interest.

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Editorial

Ivermectin and COVID-19: Keeping Rigor in Times of Urgency

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Ivermectin is a widely used drug for the treatment and control of several neglected tropical diseases.[1] The drug has an excellent safety profile, with more than 2.5 billion doses distributed in the last 30 years, and its potential to reduce malaria transmission by killing mosquitoes is under evaluation in several trials around the world.[2] Ivermectin inhibits the in vitro replication of some positive, single-stranded RNA viruses, namely, dengue virus (DNV),[3–5] Zika virus,[4,6] yellow fever virus,[7,8] and others.[4,7,9]

Caly et al.[10] recently reported that ivermectin is a potent inhibitor of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replication in vitro. Given the coronavirus disease-19 (COVID-19) pandemic, this has understandably resonated widely in the global press.[11]

Caly et al.[10] report a 5,000-fold reduction in SARS-CoV-2 RNA levels, compared with those in controls, after infected Vero/hSLAM cells were incubated for 48 hours with 5 μM ivermectin. The ivermectin IC_{50} for the virus was calculated at approximately 2.5 μM. These concentrations are the equivalent of 4,370 and 2,190 ng/mL, respectively, notably 50- to 100-fold the peak concentration (C_{max}) achieved in plasma after the single dose of 200 μg/kg (14 mg in a 70-kg adult) commonly used for the control of onchocerchiasis.[12] Pharmacokinetic studies in healthy volunteers have suggested that single doses up to 120 mg of ivermectin can be safe and well tolerated.[13] However, even with this dose, which is 10-fold greater than those approved by the US Food and Drug Administration, the C_{max} values reported were ~250 ng/mL,[13] one order of magnitude lower than effective in vitro concentrations against SARS-CoV-2.

These findings may seem to discourage follow-up clinical trials with ivermectin. However, some in vivo effect may be possible even if efficacious in vitro concentrations are physiologically unattainable. A recent phase III clinical trial in dengue patients in Thailand, in which a once-daily dose of 400 μg/kg for 3 days was found to be safe but did not produce any clinical benefit,[14] showed a modest and indirect in vivo effect against DNV.[14] Previous work by Wagstaff et al.[5] reported inhibition at much higher in vitro concentrations (25 μM) in DNV-infected Vero cells. Both pharmacokinetic considerations and the relatively long incubation period of DNV might explain the lack of clinical efficacy. Until we have a better understanding of ivermectin's antiviral mode of action and of appropriate in vitro systems for testing, we caution against using findings in Vero cells as more than a qualitative indicator of potential efficacy.

Very recently, preliminary findings on a potential effect of hydroxychloroquine combined with azithromycin against SARS-CoV-2 were widely publicized,[15] leading to a surge in demand and self-medication, which resulted in serious harm in some cases and a stock shortage that jeopardized drug availability for other critical conditions for which hydroxychloroquine or chloroquine is the standard of care, that is, vivax malaria, rheumatoid arthritis, and systemic lupus erythematosus. Efficacy claims for hydroxychloroquine against COVID-19 have been questioned in follow-up trials using similar dosing regimens,[16,17] and we await results of randomized, controlled clinical trials exploring treatment efficacy.

We believe the recent findings regarding ivermectin warrant rapidly implemented controlled clinical trials to assess its efficacy against SARS-CoV-2. These trials may open a new field of research on the potential use of avermectin antiparasitic drugs, including compounds with an improved pharmacokinetic profile, as antivirals.[18] However, because of the following points, extreme due diligence and regulatory review are needed before testing ivermectin in severe disease.

First, ivermectin, which targets glutamate-gated chlorine channels in invertebrates, may cross-target the GABA-gated chlorine channels present in the mammalian central nervous system (CNS) and cause neurotoxicity.[19] This is normally prevented by an intact blood–brain barrier (BBB), but in patients with a hyperinflammatory state, endothelial permeability at the BBB may be increased and cause leaking of drugs into the CNS, potentially causing harm.[20,21]

Second, boosted antiretrovirals such as lopinavir/ritonavir and darunavir/cobicistat, which have been widely used against SARS-CoV-2 based on limited evidence, and a number of other drugs, are potent inhibitors of cytochrome P_{450} 3A4, the main metabolic pathway for ivermectin. Concurrent use of these drugs will result in increased systemic exposure to ivermectin. Furthermore, ritonavir and cobicistat can readily inhibit one of the main efflux pumps in the BBB, P-glycoprotein, further favoring neurotoxicity.[22,23] However, it is encouraging that a recent analysis of ivermectin-related neurotoxic adverse events reported to the WHO Program for International Drug Monitoring found only one case of 1,668 reports in which concomitant use of antivirals was associated with neurotoxicity.[24]

Third, as earlier, available evidence suggests that levels of ivermectin with meaningful activity against SARS-CoV-2 would not be achieved without extraordinary, potentially toxic increases in ivermectin dosing levels in humans. However, evidence from animal models showing up to 3-fold higher levels in pulmonary tissue than in plasma 1 week after oral dosing leaves the door open for further research, in particular for the treatment of respiratory viruses.[25,26]

The discovery of ivermectin's activity against SARS-CoV-2 gives reason for hope, but off-label and compassionate use requires careful risk–benefit considerations,[27] especially in

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critically ill patients. A path to consider is evaluation first of impacts on virologic outcomes in uncomplicated, low-risk patients early in the course of the disease. Well-conducted clinical trials informed by robust pharmacokinetic models should be considered to validate the impact before the use of ivermectin to treat SARS-CoV-2 is implemented.

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Journal Pre-proof

The FDA-approved Drug Ivermectin inhibits the replication of SARS-CoV-2 *in vitro*

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The FDA-approved Drug Ivermectin inhibits the replication of SARS-CoV-2 *in vitro.*

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Summary

Although several clinical trials are now underway to test possible therapies, the worldwide response to the COVID-19 outbreak has been largely limited to monitoring/containment. We report here that Ivermectin, an FDA-approved anti-parasitic previously shown to have broad-spectrum anti-viral activity *in vitro*, is an inhibitor of the causative virus (SARS-CoV-2), with a single addition to Vero-hSLAM cells 2 hours post infection with SARS-CoV-2 able to effect ~5000-fold reduction in viral RNA at 48 h. Ivermectin therefore warrants further investigation for possible benefits in humans.

Ivermectin is an FDA-approved broad spectrum anti-parasitic agent[1] that in recent years we, along with other groups, have shown to have anti-viral activity against a broad range of viruses[2-5] *in vitro*. Originally identified as an inhibitor of interaction between the human immunodeficiency virus-1 (HIV-1) integrase protein (IN) and the importin (IMP) α/β1 heterodimer responsible for IN nuclear import[6], Ivermectin has since been confirmed to inhibit IN nuclear import and HIV-1 replication[5]. Other actions of ivermectin have been reported[7], but ivermectin has been shown to inhibit nuclear import of host (eg. [8, 9]) and viral proteins, including simian virus SV40 large tumour antigen (T-ag) and dengue virus (DENV) non-structural protein 5[5, 6]. Importantly, it has been demonstrated to limit infection by RNA viruses such as DENV 1-4[4], West Nile Virus[10], Venezuelan equine encephalitis virus (VEEV)[3] and influenza[2], with this broad spectrum activity believed to be due to the reliance by many different RNA viruses on IMPα/β1 during infection[11, 12]. Ivermectin has similarly been shown to be effective against the DNA virus pseudorabies virus (PRV) both *in vitro* and *in vivo*, with ivermectin treatment shown to increase survival in PRV-infected mice[13]. Efficacy was not observed for ivermectin against Zika virus (ZIKV) in mice, but the authors acknowledged that study limitations justified re-evaluation of ivermectin's anti-ZIKV activity[14]. Finally, ivermectin was the focus of a phase III clinical trial in Thailand in 2014-2017, against DENV infection, in which a single daily oral dose was observed to be safe and resulted in a significant reduction in serum levels of viral NS1 protein, but no change in viremia or clinical benefit was observed (see below)[15].

The causative agent of the current COVID-19 pandemic, SARS-CoV-2, is a single stranded positive sense RNA virus that is closely related to severe acute respiratory syndrome coronavirus (SARS-CoV). Studies on SARS-CoV proteins have revealed a potential role for IMPα/β1 during infection in signal-dependent nucleocytoplasmic shutting of the SARS-CoV Nucleocapsid protein[16-18], that may impact host cell division[19, 20]. In addition, the SARS-CoV

accessory protein ORF6 has been shown to antagonize the antiviral activity of the STAT1 transcription factor by sequestering IMPα/β1 on the rough ER/Golgi membrane[21]. Taken together, these reports suggested that ivermectin's nuclear transport inhibitory activity may be effective against SARS-CoV-2.

To test the antiviral activity of ivermectin towards SARS-CoV-2, we infected Vero/hSLAM cells with SARS-CoV-2 isolate Australia/VIC01/2020 at an MOI of 0.1 for 2 h, followed by the addition of 5 μM ivermectin. Supernatant and cell pellets were harvested at days 0-3 and analysed by RT-PCR for the replication of SARS-CoV-2 RNA (**Fig. 1A/B**). At 24 h, there was a 93% reduction in viral RNA present in the supernatant (indicative of released virions) of samples treated with ivermectin compared to the vehicle DMSO. Similarly a 99.8% reduction in cell-associated viral RNA (indicative of unreleased and unpackaged virions) was observed with ivermectin treatment. By 48h this effect increased to an ~5000-fold reduction of viral RNA in ivermectin-treated compared to control samples, indicating that ivermectin treatment resulted in the effective loss of essentially all viral material by 48 h. Consistent with this idea, no further reduction in viral RNA was observed at 72 h. As we have observed previously[3-5], no toxicity of ivermectin was observed at any of the timepoints tested, in either the sample wells or in parallel tested drug alone samples.

To further determine the effectiveness of ivemectin, cells infected with SARS-CoV-2 were treated with serial dilutions of ivermectin 2 h post infection and supernatant and cell pellets collected for real-time RT-PCR at 48 h (**Fig. 1C/D**). As above, a >5000 reduction in viral RNA was observed in both supernatant and cell pellets from samples treated with 5 μM ivermectin at 48 h, equating to a 99.98% reduction in viral RNA in these samples. Again, no toxicity was observed with ivermectin at any of the concentrations tested. The IC50 of ivermectin treatment was determined to be ~2μM under these conditions. Underlining the fact that the assay indeed specifically detected SARS-CoV-2, RT-PCR experiments were

repeated using primers specific for the viral RdRp gene (**Fig. 1E/F**) rather than the E gene (above), with nearly identical results observed for both released (supernatant) and cell-associated virus.

Taken together these results demonstrate that ivermectin has antiviral action against the SARS-CoV-2 clinical isolate *in vitro*, with a single dose able to control viral replication within 24-48 h in our system. We hypothesise that this is likely through inhibiting IMPα/β1-mediated nuclear import of viral proteins (**Fig. 1G**), as shown for other RNA viruses [4, 5, 10]; confirmation of this mechanism in the case of SARS-CoV-2, and identification of the specific SARS-CoV-2 and/or host component(s) impacted (see [10]) is an important focus future work in this laboratory. Ultimately, development of an effective anti-viral for SARS-CoV-2, if given to patients early in infection, could help to limit the viral load, prevent severe disease progression and limit person-person transmission. Benchmarking testing of ivermectin against other potential antivirals for SARS-CoV-2 with alternative mechanisms of action[22-26] would thus be important as soon as practicable. This Brief Report raises the possibility that ivermectin could be a useful antiviral to limit SARS-CoV-2, in similar fashion to those already reported[22-26]; until one of these is proven to be beneficial in a clinical setting, all should be pursued as rapidly as possible.

Ivermectin has an established safety profile for human use[1, 12, 27], and is FDA-approved for a number of parasitic infections[1, 27]. Importantly, recent reviews and meta-analysis indicate that high dose ivermectin has comparable safety as the standard low-dose treatment, although there is not enough evidence to make conclusions about the safety profile in pregnancy [28, 29]. The critical next step in further evaluation for possible benefit in COVID-19 patients will be to examine a multiple addition dosing regimen that mimics the current approved usage of ivermectin in humans. As noted, ivermectin was the focus of a recent phase III clinical trial in dengue patients in Thailand, in which a single daily dose was found

to be safe but did not produce any clinical benefit. However, the investigators noted that an improved dosing regimen might be developed, based on pharmacokinetic data[15]. Although DENV is clearly very different to SARS-CoV-2, this trial design should inform future work going forward. Altogether the current report, combined with a known-safety profile, demonstrates that ivermectin is worthy of further consideration as a possible SARS-CoV-2 antiviral.

Methods

Cell culture, viral infection and drug treatment

Vero/hSLAM cells[30] were maintained in Earle's Minimum Essential Medium (EMEM) containing 7% Fetal Bovine Serum (FBS) (Bovogen Biologicals, Keilor East, AUS) 2 mM L-Glutamine, 1 mM Sodium pyruvate, 1500 mg/L sodium bicarbonate, 15 mM HEPES and 0.4 mg/ml geneticin at 37°C, 5% CO_2. Cells were seeded into 12-well tissue culture plates 24 h prior to infection with SARS-CoV-2 (Australia/VIC01/2020 isolate) at an MOI of 0.1 in infection media (as per maintenance media but containing only 2% FBS) for 2 h. Media containing inoculum was removed and replaced with 1 mL fresh media (2% FBS) containing Ivermectin at the indicated concentrations or DMSO alone and incubated as indicated for 0-3 days. At the appropriate timepoint, cell supernatant was collected and spun for 10 min at 6,000g to remove debris and the supernatant transferred to fresh collection tubes. The cell monolayers were collected by scraping and resuspension into 1 mL fresh media (2% FBS). Toxicity controls were set up in parallel in every experiment on uninfected cells.

Generation of SARS-CoV-2 cDNA

RNA was extracted from 200 μL aliquots of sample supernatant or cell suspension using the QIAamp 96 Virus QIAcube HT Kit (Qiagen, Hilden, Germany) and eluted in 60 μl. Reverse

transcription was performed using the BioLine SensiFAST cDNA kit (Bioline, London, United Kingdom), total reaction mixture (20 µl), containing 10 µL of RNA extract, 4 µl of 5x TransAmp buffer, 1µl of Reverse Transcriptase and 5 µl of Nuclease free water. The reactions were incubated at 25°C for 10 min, 42°C for 15 min and 85°C for 5 min.

Detection of SARS-CoV-2 using a TaqMan Real-time RT-PCR assay.

TaqMan RT-PCR assay were performed using 2.5 µl cDNA, 10 µl Primer Design PrecisonPLUS qPCR Master Mix 1 µM Forward (5'- AAA TTC TAT GGT GGT TGG CAC AAC ATG TT-3'), 1 µM Reverse (5'- TAG GCA TAG CTC TRT CAC AYT T-3') primers and 0.2 µM probe (5'-FAM- TGG GTT GGG ATT ATC-MGBNFQ-3') targeting the BetaCoV RdRp (RNA-dependent RNA polymerase) gene or Forward (5'-ACA GGT ACG TTA ATA GTT AAT AGC GT -3'), 1 µM Reverse (5'-ATA TTG CAG CAG TAC GCA CAC A-3') primers and 0.2 µM probe (5'-FAM-ACA CTA GCC ATC CTT ACT GCG CTT CG-

286 NFQ-3') targeting the BetaCoV E-gene[31]. Real-time RT-PCR assays were performed on an Applied Biosystems ABI 7500 Fast real-time PCR machine (Applied Biosystems, Foster City, CA, USA) using cycling conditions of 95°C for 2 min, 95°C for 5 s, 60°C for 24 s. SARS-CoV-2 cDNA (Ct~28) was used as a positive control. Calculated Ct values were converted to fold-reduction of treated samples compared to control using the ΔCt method (fold changed in viral RNA = 2^ΔCt) and expressed as % of DMSO alone sample. IC50 values were fitted using 3 parameter dose response curves in GraphPad prism.

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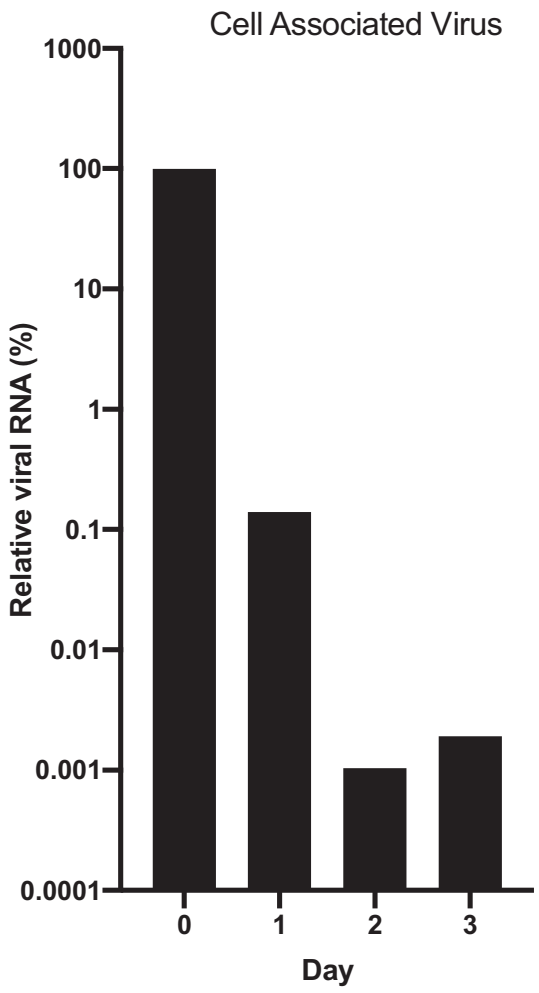
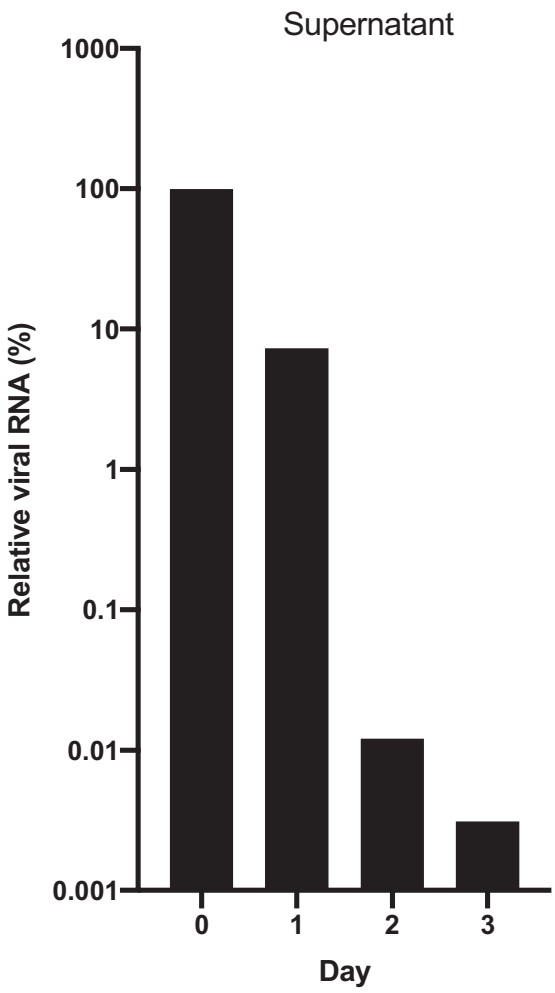
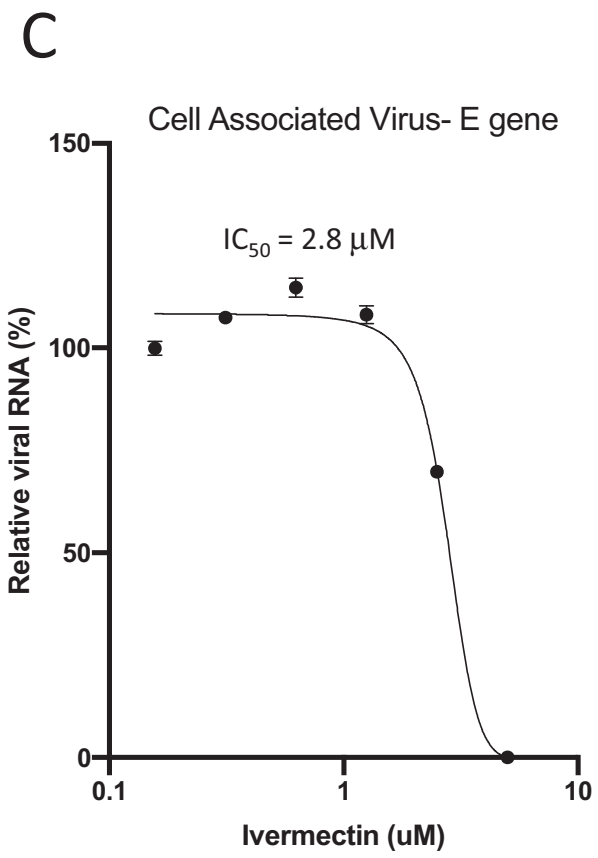
Figure 1: Ivermectin is a potent inhibitor of the SARS-CoV-2 clinical isolate Australia/VIC01/2020. Vero/hSLAM cells were in infected with SARS-CoV-2 clinical isolate Australia/VIC01/2020 (MOI = 0.1) for 2 h prior to addition of vehicle (DMSO) or Ivermectin at the indicated concentrations. Samples were taken at 0-3 days post infection for quantitation of viral load using real-time PCR of cell associated virus (**A**) or supernatant (**B**). IC_{50} values were determined in subsequent experiments at 48 h post infection using the indicated concentrations of Ivermectin (treated at 2 h post infection as per **A/B**). Triplicate real-time PCR analysis was performed on cell associated virus (**C/E**) or supernatant (**D/F**) using probes against either the SARS-CoV-2 E (**C/D**) or RdRp (**E/F**) genes. Results represent mean ± SD (n=3). 3 parameter dose response curves were fitted using GraphPad prism to determine IC_{50} values (indicated). **G.** Schematic of ivermectin's proposed antiviral action on coronavirus. IMPα/β1 binds to the coronavirus cargo protein in the cytoplasm (top) and translocates it through the nuclear pore complex (NPC) into the nucleus where the complex falls apart and the viral cargo can reduce the host cell's antiviral response, leading to enhanced infection. Ivermectin binds to and destabilises the Impα/β1 heterodimer thereby preventing Impα/β1 from binding to the viral protein (bottom) and preventing it from entering the nucleus. This likely results in reduced inhibition of the antiviral responses, leading to a normal, more efficient antiviral response.

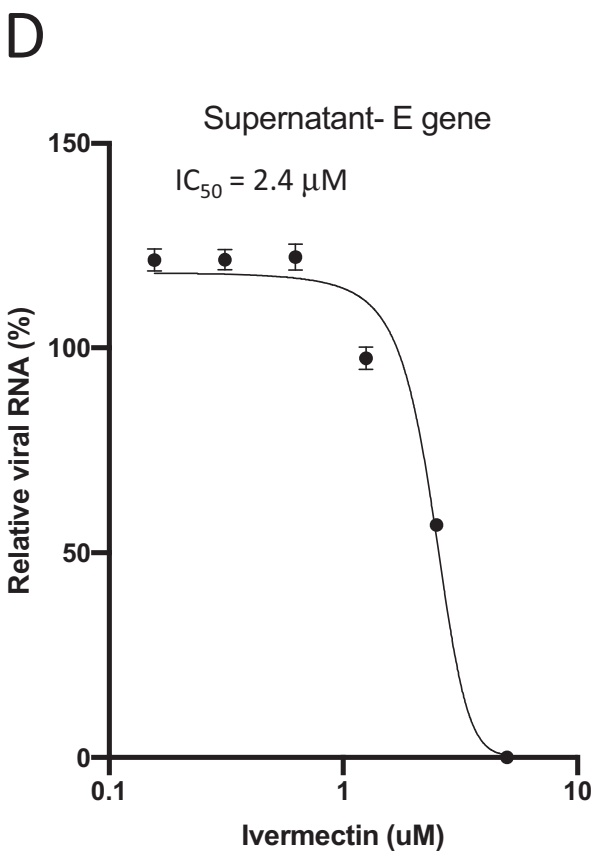
A

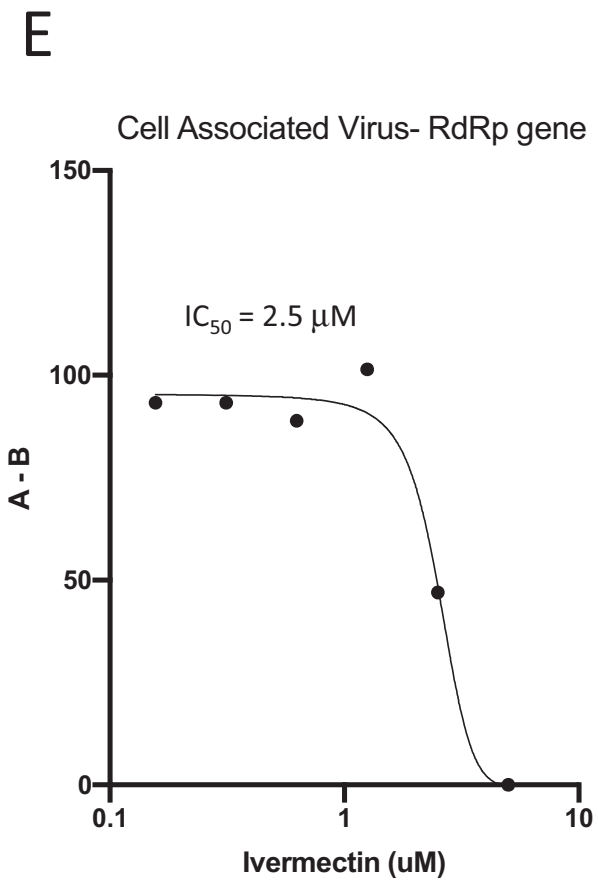

Cell Associated Virus
1000
100
10
1
0.1
0.01
0.001
0.0001
Relative viral RNA (%)
0
1
2
3
Day

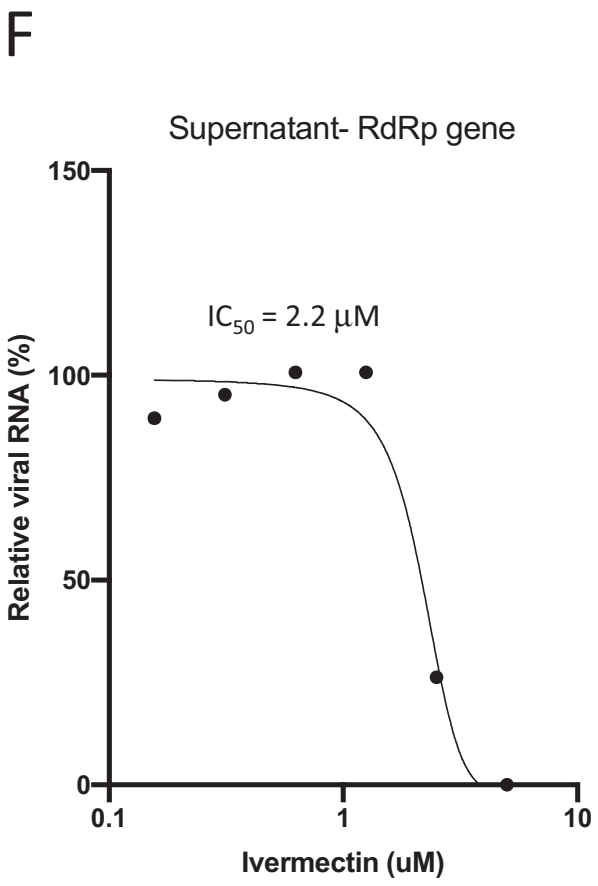
B


Supernatant
1000
100
10
1
0.1
0.01
0.001
Relative viral RNA (%)
0
1
2
3
Day

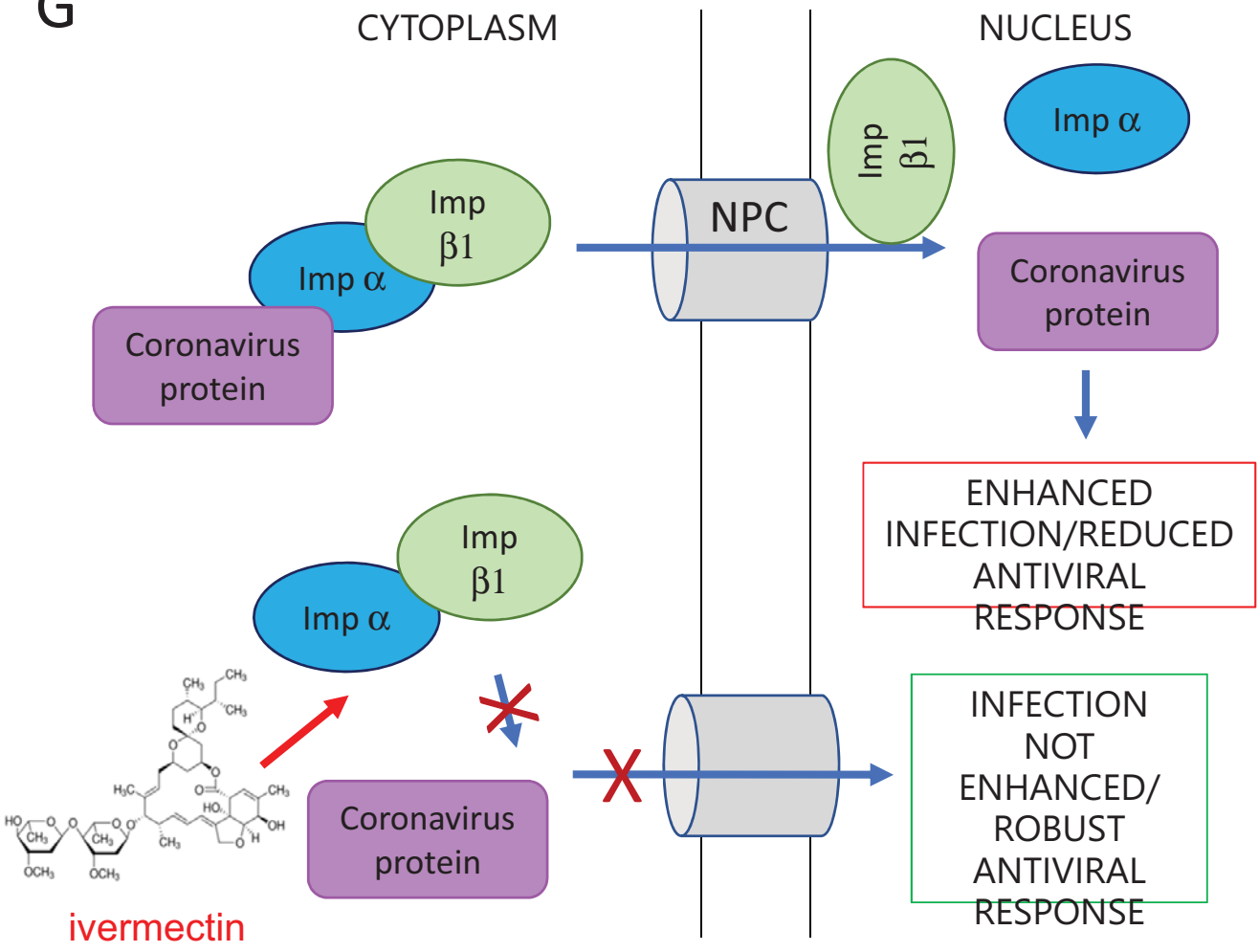

C
Cell Associated Virus- E gene
IC50 = 2.8 µM
Relative viral RNA (%)
150
100
50
0
0.1
1
10
Ivermectin (uM)


D
Supernatant- E gene
IC50 = 2.4 µM
Relative viral RNA (%)
150
100
50
0
0.1
1
10
Ivermectin (uM)


E
Cell Associated Virus- RdRp gene
IC50 = 2.5 µM
A - B
150
100
50
0
0.1
1
10
Ivermectin (uM)


F
Supernatant- RdRp gene
IC50 = 2.2 µM
Relative viral RNA (%)
150
100
50
0
0.1
1
10
Ivermectin (uM)

G


CYTOPLASM
NUCLEUS
Imp β1
Imp α
NPC
Imp β1
Imp α
Coronavirus protein
Coronavirus protein
ENHANCED INFECTION/REDUCED ANTIVIRAL RESPONSE
Imp β1
Imp α
Coronavirus protein
INFECTION NOT ENHANCED/ ROBUST ANTIVIRAL RESPONSE
ivermectin

Highlights

- Ivermectin is an inhibitor of the COVID-19 causative virus (SARS-CoV-2) *in vitro.*
- A single treatment able to effect ~5000-fold reduction in virus at 48h in cell culture.
- Ivermectin is FDA-approved for parasitic infections, and therefore has a potential for repurposing.
- Ivermectin is widely available, due to its inclusion on the WHO model list of essential medicines.