The selective effect of Ivermectin on different human coronaviruses; in-vitro study

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Article

Keywords: Coronavirus, Alphacorona, Betacorona, SARS-CoV-2, Omicron, Severe acute respiratory syndrome, Ivermectin

Posted Date: April 18th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4180797/v1

Additional Declarations: No competing interests reported.

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Background: The outbreak of coronavirus disease COVID-19, caused by Severe Acute Respiratory Coronavirus-2 (SARS-CoV-2) has become an urgent public health concern worldwide. Although several clinical trials have pointed to new drugs with some anti-COVID-19 activity, we are far from having a safe and effective drug. In this study, we tested the effect of ivermectin on several coronaviruses (serotypes), including variants of SARS-CoV-2.

Methods: The effect of ivermectin was tested on cells infected with four different coronaviruses: NL63 (Alphacoronavirus genus.), OC43, SARS-CoV-2, and Omicron (all Betacoronavirus genus). Two hours post-infection, different doses of ivermectin were added to the cell culture.

Results: There was no effect of even a high dose of ivermectin on NL63, however, we found a significant effect on OC43 PFU with a 40% inhibition at a dose of 5μM. The impact of ivermectin on SARS-CoV-2 and on its Omicron variant was much more pronounced and at a dose of 5μM there was inhibition of 90% and 95% respectively.

Discussion: Although coronaviruses have been recognized as human pathogens for more than 50 years, no effective treatment strategy exists. Our current study did not demonstrate any effect of ivermectin on Alphacoronavirus but it had a specific impact on the Betacoronavirus genus with a mild impact on OC43 and a decidedly pronounced effect on SARS-CoV-2 including its Omicron variant. Ivermectin should be further studied as a single agent or as part of combined treatment against Coronaviruses.

Keywords: Coronavirus, Alphacorona, Betacorona, SARS-CoV-2, Omicron, Severe acute respiratory syndrome, Ivermectin.

1. Introduction

Coronaviruses (CoVs) belong to a family of enveloped positive-sense single-stranded RNA viruses that are distributed broadly among humans, mammals, and birds, and cause respiratory, enteric, hepatic, and neurologic diseases [1]. Six coronavirus species are known to cause human disease. Four viruses, 229E, OC43, NL63, and HKU1, are prevalent and typically cause common cold symptoms in immunocompetent individuals [1]. The two other strains cause the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus (MERS-CoV). They are zoonotic in origin and have been associated with a case fatality rate (CFR) estimated at 15 % in SARS-CoV and 43% in the case of MERS-Cov [2]. At the end of 2019, an outbreak of severe acute respiratory syndrome (the COVID-19 pandemic), caused by a new member of the Beta coronaviruses, SARS-CoV-2, was reported in China and spread throughout the world. Given the high prevalence and wide distribution of corona viruses, the large genetic diversity and genomic mutation rate, as well as an increase in human-animal interface activities, novel coronaviruses are likely to emerge periodically in humans due to frequent cross-species infections and occasional "spillover" events [3,4].

Two genera within the coronavirus's family infect humans: The Alpha coronavirus, and the Beta coronavirus. NL63 (HCoV-NL63) phylogenetically clusters within the genus *Alphacoronavirus.* OC43 (CoV-OC43) clusters within Beta coronaviruses [Fig 1]. The beta coronaviruses comprise a large number of mammal-infecting viruses, as well as the five human pathogens mentioned above, i.e. HCoV-OC43, HCoV-HKU1, and the three viruses SARS-CoV -1, MERS-CoV- and SARS- CoV-2.

Finding anti-coronavirus drugs **has** become an important undertaking. Currently, there are three drugs that have been FDA-approved as antiviral agents to treat SARS-cov-2 infect

ion, namely Paxlovid (Ritonavir-Boosted Nirmatrelvir**)** [5], Lagevrio (Molnupiravir) [6], both given orally and Veklury (remdesivir) [7] which is given parenterally. These drugs have limited efficacy and several limitations. Therefore, finding a drug or drug combination which might be more effective and well-tolerated is essential.

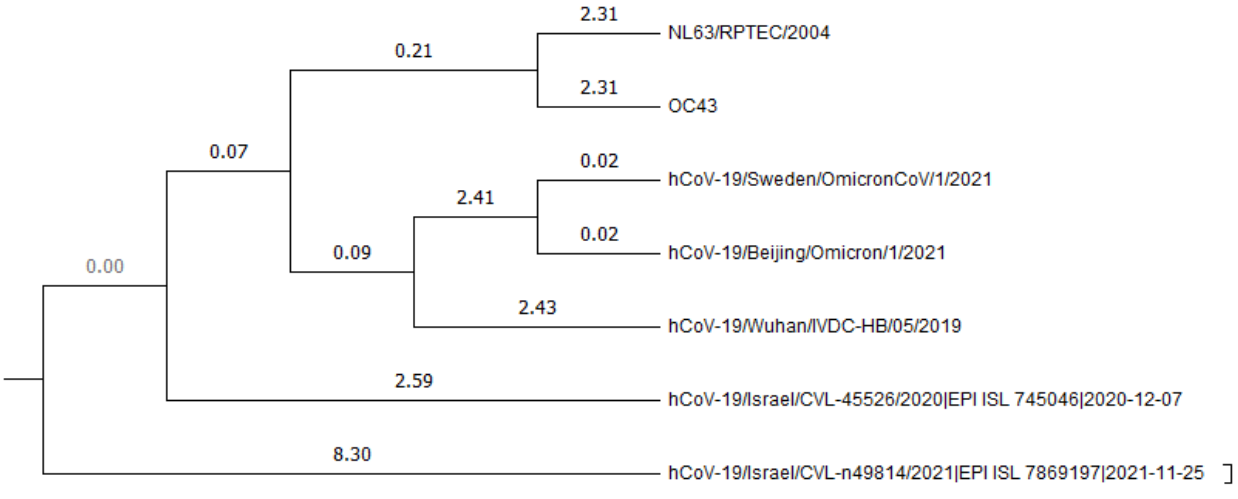

Fig 1: Phylogenetic tree of the coronaviruses used in this study. The phylogenetic tree was performed using The Molecular Evolutionary Genetics Analysis (MEGA) software [8]. * From the coronaviruses family in this diagram, hCoV-NL63 is the only one that belongs to the Alphacoronavirus. The number above each line in the phylogenetic tree represents the Evolutionary distance calculated according to the difference between the sequences of each virus. The smaller the number implies a smaller difference between the sequences of the different viruses [9,10].

Ivermectin's chemical structure consists of a homologues mixture of 5-O-dimethyl-22,23-dihydroavermectin B1a (80%) and B1b (20%) [11]. The drug **is a broad-spectrum** anti-microbial agent approved by the FDA to treat several parasitic infections [12,13]. In-vitro studies have shown its anti-viral activity against a wide **range of** RNA **viruses** such as hepatitis E [14], foot-and-mouth disease virus [15], dengue virus [16], and bovine respiratory viruses [17]. Ivermectin was also shown to strongly inhibit nuclear-replicating DNA viruses such as Herpesviruses [18], Parvoviruses [19], Polyomaviruses [20], and adenoviruses [21]. Additional viruses effected by Ivermectin reviewed in Patil, VM et al. [22]. Caly *et al.* reported its specific activity against SARS-CoV-2 in cell culture and suggested its broad-spectrum activity might be due to its activity as a small molecule inhibitor of importin (IMP) superfamily, α and β forms (IMPα/β1). IMPα/β1 complex pathway is one of the pathways by which proteins enter the nucleus. Various RNA viruses such as the human immune deficiency virus-1 (HIV-1), dengue, and Zika, are dependent on IMPα/β1 nuclear transport activity for infection. Hence, small molecule inhibitors of IMPα/β1, such as ivermectin could be used as an anti-viral drug, as reviewed in [23] and [12,13,24]. However, with the increased interest in this drug as an anti-viral agent, many other modes of action have been proposed [22]. As mentioned above, parts of the virus proteins are transported to the cell nucleus by binding to the IMPα/ß1 complex. In the nucleus, virus protein reduces the host cell's anti-viral response. The hypothesis is that ivermectin inhibits the binding of the virus proteins to the IMPα/ß1 complex and subsequently allows an immune-host response [22]. Another mode of action that has been proposed is the ability of ivermectin to bind to the viral spike receptor binding domain at the ACE2 receptor, which might interfere with the attachment of the virus to the cell membrane [25]. Ivermectin was also found to have the ability to bind to the predicted active site of SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) [26]. Other modes of action were summarized in a number of review papers [27,28]]. A limitation of the study of Caly *et al.* which showed the significant activity of ivermectin against SARS-CoV-2 in cell culture was the necessity of a high dose of ivermectin to demonstrate its in-vitro anti-SARS-CoV-2 activity [12]. In addition, the study was performed at a very

early stage during the pandemic and therefore was only tested against the wild type of the virus. Clinical studies during the COVID pandemic gave conflicting results on the efficacy of the drug and fueled an international debate. Meta-analyses that were performed did not resolve the argument but rather perpetuated it [29].

Our study sought to test the antiviral activity of ivermectin in infected cell cultures of several human coronaviruses, including alpha corona and beta coronaviruses, the SARS-CoV-2 including its new Omicron variant.

2. Materials and Methods

Cell cultures

Vero E6 cells (African green monkey kidney cells) pusher from ATCC catalog number CRL-1586, were grown in MEM-EAGLE Earle's Salts Base (Biological Industries) medium supplemented with 10% fetal bovine serum (FBS, ThermoFisher Scientific), 100 units/ml penicillin (ThermoFisher Scientific) and 100 μg/ml streptomycin (ThermoFisher Scientific) at 37 °C with 5% CO_2. Since the ability of the NL-63 virus to infect VERO-E6 cells is limited [30], we used CaCo-2 cells for this virus.

CaCo-2 cells (Human colon carcinoma cells) pusher from ATCC catalog number HTB-37, were grown in Dulbecco's Modified Eagle Medium (Biological Industries) supplemented with 20% FBS or EVs-depleted FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C with 5% CO_2.

Viruses

HCoV- OC43 virus was purchased from ATCC (VR-1558) and was propagated in Vero E6 cells [31]. The supernatant from the infected cells was aliquoted and stored at -80°C.

The HCoV-NL63 virus used in this study was obtained from BEI Resources (Catalog No. NR-470). HCoV-NL63 was propagated in CaCo-2 cells [30].

SARS-CoV-2 (hCoV19/Israel/CVL-45526-ngs/2020) and Omicron SARS-CoV-2 (BA.1 hCoV-19/Israel/CVL-n49814/2021).

Viruses were isolated from positive nasopharyngeal swab samples.,. The viruses were propagated in Vero E6 cells [32]. Our viral stocks did not undergo more than two passages. We used the following protocol: we collected nasopharyngeal samples from SARS-CoV-2 positive individuals [which contained the Wuhan strain (hCoV-19/Israel/CVL-45526-ngs/2020),]. Confluent VERO-E6 cells were incubated for 1 hour at 33ºC with 300 μl of the nasopharyngeal samples, followed by the addition of 5 ml 2% FCS MEM-EAGLE medium. When Cytopathic effect (CPE) was observed, 300 μl of the supernatant was taken and added to a previously prepared T-75 flask seeded with VERO-E6 cells. The flasks were immediately filled with 20 ml 2% FCS MEM-EAGLE and incubated at 33ºC in order to reach higher viral loads as detailed in Lustig, Y. et al.[33]. The supernatant from the infected cells was aliquoted and stored at -80°C. We were aware of the possibility of mutations as a result of passages in cell culture, specifically the furin cleavage site [34]. Hence, 200 μl from the supernatants were taken for nucleic acids extraction followed by sequencing. In both SARS-CoV-2 (hCoV19/Israel/CVL-45526-ngs/2020) and Omicron SARS-CoV-2 (BA.1 hCoV-19/Israel/CVL-n49814/2021) the furin cleavage site maintained the same sequence.

In vitro Virus infection and ivermectin treatment.

To test the effect of the antiviral activity of ivermectin on HCoV OC43 and NL63, 2X10[4] Vero E6 cells, and CaCo-2 cells were seeded in 96-well plates. Twenty-four hours after the seed, the cells were infected with 0.01 MOI virus. The cells were incubated with the viruses for two hours followed by the addition of 1, 2.5, 5, and 10μM of Ivermectin. Seventy-two hours after infection, to check the viral RNA amount of the viruses released from the infected cells, the supernatant was collected. NL63 infections were made in CaCo-2 because the ability of NL-63 to infect VERO-E6 cells is not optimal [30].

RNA purification and quantification

Viral RNA was extracted from cell culture supernatant using the Norgen Biotek Total RNA Purification Kit (Cat. # 17200) in accordance with the manufacturer's instructions. Quantitative real-time RT-PCR was performed on a Quant Studio 6 Flex (ABI, Foster City, CA, USA) instrument, using TaqMan™ Fast Advanced Master Mix (Thermo Fisher Scientific, Cat# 4444558). Ct values were converted to the RNA virus particles by generating calibration curves for the envelope protein (E), nucleocapsid protein (N), and RNA-dependent RNA polymerase (RdRp) qPCR reactions, as commonly utilized in many studies. The quantification cycle (Cq) values obtained from the standard calibration qRT-PCR assays were plotted against each RNA target's measured and calculated concentration.

,[see details in the Supplementary material, in S5 Fig and S1-S4 Tables, in Supplementary data]. The primers were designed so that they would be adapted to several strains of the SARS Betacoronavirus We used values obtained from the standard calibration qRT-PCR assays of the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) mRNA [S5 Fig and S4 Table].

The primers and probes sequences are listed below:

HCoV-NL63

Forward: 5′ GACCAAAGCACTGAATAACATTTTCC 3′

Reverse: 5′ACCTAATAAGCCTCTTTCTCAACCC 3′

Probe[c]: 5′ AACACGCT"T"CCAACGAGGTTTCTTCAACTGAG 3′

HCoV-OC43

Forward: 5′ CGATGAGGCTATTCCGACTAGGT 3′

Reverse: 5′ CCTTCCTGAGCCTTCAATATAGTAACC 3′

Probe[b]: 5′ TCCGCCTGGCACGGTACTCCCT 3′

SARS CoV-2

Detection of SARS-CovV-2 (SC-2) RNA was performed using a combination of reactions developed by Corman *et al.* [35] and the Centers for Disease Control and Prevention (CDC) (https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html), with some modifications, as follows:

E gene reaction

Forward: 5′GTTAATAGCGTACTTCTTTTTCTTGC 3′

Reverse: 5′ATATTGCAGCAGTACGCACACA 3′

Probe: 5′-6-FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ1- 3′

N gene reaction

Forward: 5′-CTAAACGAACAAACTAAAATGTCTG 3′

Reverse: 5′ TCTGGTTACTGCCAGTTGAATCTG 3′

Probe: 5′ HEX-ACCCCGCATTACGTTTGGTGGACC-BHQ1 3′

Human RNAse P gene reaction

Forward: 5′-AGATTTGGACCTGCGAGCG

Reverse: 5′ GAGCGGCTGTCTCCACAAGT

Probe: 5′ Cy5-TTCTGACCTGAAGGCTCTGCGCG-BHQ2 – 3′

We confirm that all methods were carried out in accordance with relevant guidelines and regulations.

All experimental protocols were approved by Sheba Medical Center Institutional Review Board, approval number 7875-20-SMC.

Preparation of ivermectin

The ivermectin was provided by Super-Pharm Israel. The ivermectin was solubilized in DMSO at a stock concentration of 1M and then diluted in medium (EMEM or DMEM) to make a working solution of 1, 2.5, 5, and 10 μM.

Inhibition of HCoV infection using ivermectin

A culture of $2X10^4$ Vero E6 cells and CaCo-2 cells were seeded into 96-well plates twenty-four hours before infection. Infection with HCoV: The cells were infected with the indicated HCoV viruses (HCoV-OC43 and HCoV-NL63) at 0.01 MOI for 2 hours at 33 °C or 34 °C (for NL63 virus), 5% CO_2. Infection with SARS-CoV-2: Vero E6 cells were infected with WT SARS-CoV-2 and Omicron SARS-CoV-2 (BA.1 hCoV-19/Israel/CVL-n49814/2021) at a concentration of one hundred TCID50 (50% endpoint titer) for 1 hour at 33 °C.

The concentration of infection, 0.01 MOI, was calculated for HCoV-OC43 based on PFU assay on VERO-E6 cells, see S1 Fig in supplementary data and for HCoV-NL63 calculated in Caco-2 cells. This concentration of 0.01 MOI is routinely used in in-vitro studies of coronaviruses infections, as shown in [36-39].

After 2 hours, the virus was removed, cells were washed with PBS, and 50μL of fresh 2% EMEM (for Vero E6 cells) or DMEM (for Caco-2 cells) medium containing ivermectin at the indicated concentrations or clean medium as a control, was added and was incubated for 48/72 hours. After 48/72 hours, the supernatant was collected and analyzed by RT-PCR for the detection of viral RNA. We examined the reduction of viral RNA in the treated cells as compared to control samples. Toxicity controls were set up in parallel in every experiment on uninfected cells.

Statistical Analysis

Statistical tests were performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com

3. Results

To analyze whether ivermectin affects various members of the coronavirus family we have chosen two common coronaviruses; NL63 a member of the Alpha coronavirus, and OC43, a member of the Beta coronavirus. As shown in Fig 2, there was a different effect of the ivermectin on NL63 compared to OC43. In Vero-E6 cells that were infected with OC43 and treated with 2.5μM ivermectin, we found a reduction from ~1X10^5 virus particles to ~7.2X10^4 virus particles, demonstrating a significant reduction of 31% in virus particles. At a higher dose of 5μM ivermectin, a further reduction was observed from ~1X10^5 virus particles in control cells to ~5.3X10^4 virus particles in treated cells, demonstrating a significant reduction of 49% in virus particles (Fig 2A). In contrast to OC43, when Vero-E6 were infected with the NL63 virus, there was no significant decrease in the amount of virus particles (Fig 2B).

These results indicate that there is a different effect of ivermectin on the various strains of HCoVs. It has been suggested that the ability of NL-63 to infect VERO-E6 cells is not optimal [30]. Therefore, we performed the same experiment with NL63 that were carried out in CaCo-2. The results were the same as in CaCo-2 cells, ivermectin did not affect the infection of NL63 in VERO-E6 cells (supplementary data S3 Fig).

No toxicity of ivermectin was observed at concentrations of 0.5μM to 5μM. However, at a concentration of 10μM ivermectin, we observed a high cell death (Supplementary data S2 Fig)

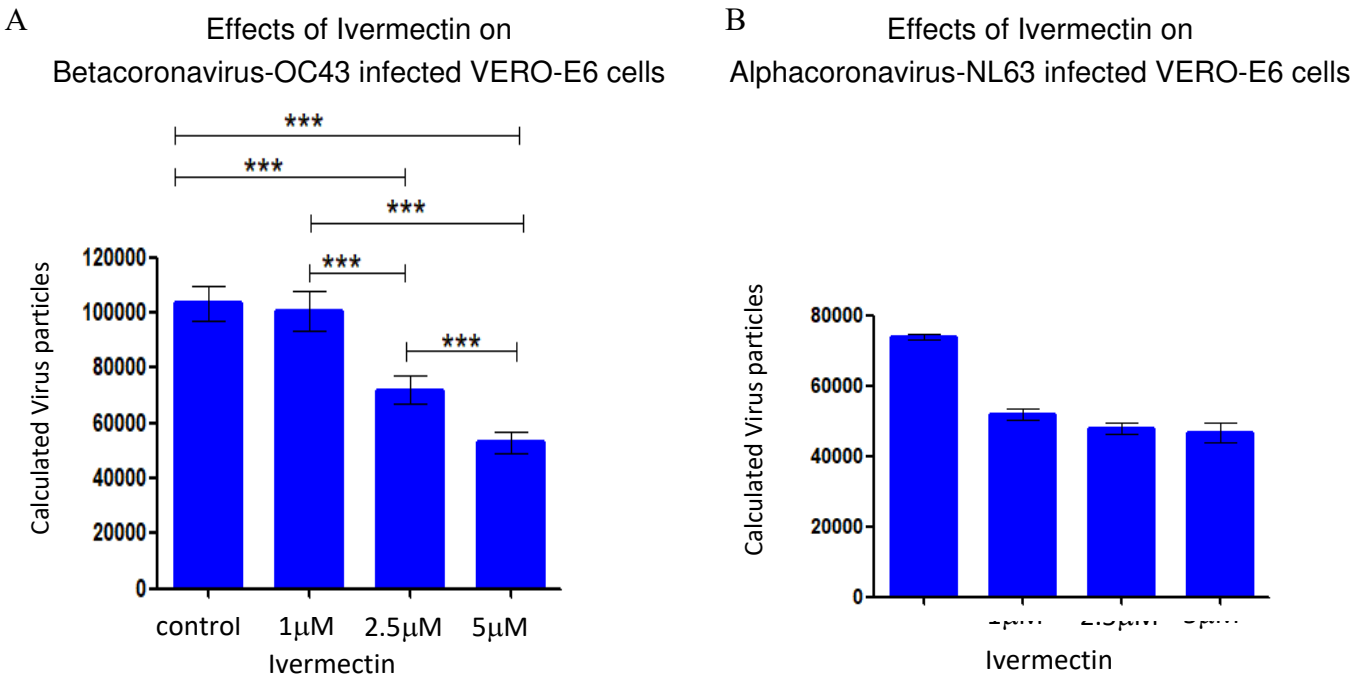

Fig 2: A) CaCo-2 cells or B) Vero E6 cells, were infected with HCoV-NL63 or HCoV-OC43, respectively, (0.01 MOI) for 2 h before the addition of a fresh medium or ivermectin at the indicated concentrations. The supernatant was collected 72h after infection, viral RNA was purified and analyzed by RT-PCR. The results represent the copy number calculation of viral RNA in treated cells compared to infected untreated cells. The graphs represent three independent experiments. Error bars show ± SEM. Statistics were performed using one-way ANOVA and Turkey's Multiple Comparison Test; ***p <0.001.

The calculation of the number of virus particles was best on the conversion of CT results of the qRT-PCR as shown in supplementary data Fig 5S.

Infection of Vero E6 cells with SARS-CoV-2

We next examined whether ivermectin affects SARS-CoV-2 infection. SARS-CoV-2 belongs to the Betacoronavirus group, like the OC43 virus. Vero E6 cells were infected with the SARS-CoV2 virus followed by an additional amount of 1, 2.5, and 5μM of Ivermectin. Forty-eight hours after infection, the supernatant was collected and analyzed by RT-PCR for the detection of viral RNA. As shown in Fig 3A, ivermectin had a dramatic and significant effect on SARS-CoV-2 infection. At a dose of 5μM, we found a reduction of 90% of the virus particles, from ~4.1X10^{7} particles to ~1X10^{3} particles. We then tested whether ivermectin had a similar effect on the Omicron variant of SARS-CoV2. As can be seen in Fig 3B, ivermectin had the same effect on the Omicron variant as the SARS-COV2 variant. A dose of 5μM of ivermectin very nearly erased the virus infection with a reduction of 95% of the virus particles from ~1X10^{9}-particles to ~1X10^{5} particles. In S4 Fig in the supplementary data, it is also apparent that after twenty-four hours ivermectin affected both the SARS-CoV-2 and Omicron SARS-CoV-2 variant.

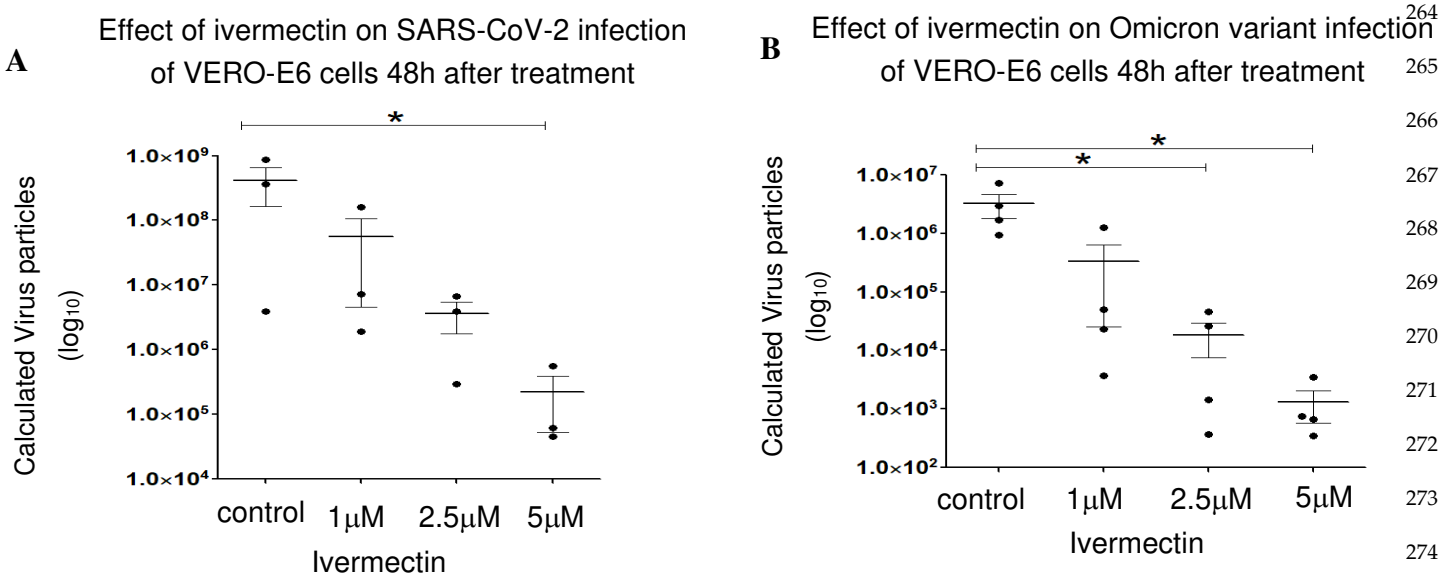

Fig 3: Vero E6 cells were infected with A) SARS-CoV2 or B) Omicron SARS-CoV-2 variant,(0.01 MOI) 2 h before the addition of a fresh medium or ivermectin at the indicated concentrations. After 48h, the supernatant was collected, and viral RNA was analyzed by RT-PCR. The results represent Calculated Virus particles in treated cells compared to infected untreated cells. Y-axis data is presented in log10 scale graphs. The graphs represent three independent experiments. Error bars show ± SEM, Median is represented as a vertical line. Statistics were performed using one-way ANOVA and Tukey's Multiple Comparison Test; *p <0.05. The calculation of the number of virus particles was best on the conversion of CT results of the qRT-PCR as shown in supplementary data Fig 5S.

4. Discussion

Coronaviruses (CoVs) belong to the subfamily Coronaviriane in the family of Coronaviride. The Coronaviridae is further specified into the subfamily of *Orthocoronavirinae*, which includes four genera: *Alphacoronavirus, Betacoronavirus, Gammacoronavirus,* and *Deltacoronavirus* [3].

Our study aimed to further characterize the effect of ivermectin on different genera of HCoVs, which, to the best of our knowledge, has never been done before. We chose NL63 as a common alphacoronavirus species and OC43 as a common betacoronavirus species. In addition, SARS-CoV-2 and the Omicron variant were studied to represent the SARS-CoV-2 strains. Our results show no detectable effect of ivermectin on an alphacoronavirus, including at a higher dose of 5μM, while the drug had a moderate effect on the common beta coronavirus OC43, mostly at doses of 2.5μM and higher. Interestingly, it seems that ivermectin has a more specific effect on the newly emerging SARS-CoV-2 pathogen. In our study, the effect was demonstrated even with a relatively low dose of 1μM which is close to the ivermectin level which is seen in treating humans. Furthermore, the drug has a similar effect on the new and currently worldwide dominant variant, the Omicron ((BA.1 hCoV-19/Israel/CVL-n49814/2021)).

The mechanism of the antiviral activity of ivermectin is considered to be related to its ability to target the host importin (IMP) $\alpha/\beta1$ nuclear transport proteins responsible for nuclear entry of cargoes of viral proteins, which in turn block the host anti-viral activity [27]. In addition, it may inhibit RNA-virus replication by interacting with RdRp, nsp14, N phosphoprotein, M protein, Mpro, PLpro, 3 chymotrypsin-like proteases and by inhibiting the KPNA/KPNB1- mediated nuclear import of viral proteins. Furthermore, it also interferes with SARS-CoV-2 cell entry by docking in binding sites of the receptor-binding domain (RBD) of the spike protein [22,25,27]. Thus, our observation that highlights the varying sensitivities of coronaviruses to the drug, with beta coronavirus being highly sensitive to ivermectin, while alpha coronavirus not being affected by the drug, may suggest a relationship to its activity on the spike protein. In fact, comparing the mapped RBD of NL63 (alpha coronavirus) [40] with the severe acute respiratory syndrome coronavirus (SARS-CoV)(, beta coronavirus) [41], using the protein-blast program [42], we found only 31% identities, which may explain this difference in sensitivity to the drug. We also found 36% identities in the comparison of the RBD of NL63 to OC43 (another beta coronavirus). Further studies are needed to elucidate whether these differences suggest an explanation for the dissimilar impact of ivermectin on alpha coronavirus compared to beta coronavirus.

Our experiments were done in-vitro on cells, which obviously limits the findings as it is hard to predict the activity in-vivo. However, the strength of our results is clear by showing a higher activity of ivermectin on SARS-CoV-2 infected cells. Moreover, the dose needed for this effect was relatively low (2.5μM), unlike the previous report from Australia where 5μM was needed [12]. Taking this into account with the higher doses of ivermectin that were used safely during the COVID epidemic (more than X10 times of the usual dose**),** our in-vitro results can be considered compatible with the in-vivo doses [43].

During the COVID-19 pandemic ivermectin was used for both treatment and prevention of COVID-19 in patients [44]. However, the clinical implication of using ivermectin in preventing hospitalization, and reducing mortality, as well as its use for prophylaxis is an ongoing debate among the medical community with some claiming that positive results were largely based on less rigorous and stringent scientific criteria [44]. An 'Ivermectin for COVID' website, collecting all randomized ivermectin studies in relation to COVID analyzed approximately **99** studies which have been published to date **[**see **https://ivmmeta.com**]. The pooled analysis showed the positive impact of the drug in the different stages of the disease. Ivermectin reduces mortality, prevents the need for artificial ventilators, decreases ICU admissions, hospitalizations, and disease progression, and supports both faster recovery and viral clearance **[https://ivmmeta.com**]. Several reviews and meta-analyses however dispute these findings and disagree with the value of this drug in treating COVID-19. Regarding the impact of the drug on mortality, few meta-analyses of ivermectin trials have strongly indicated a treatment benefit in reducing mortality, and others have concluded that there was no clear benefit [45-49]. Regarding the effect of the drug in reducing viral load, our clinical study on the effect of three days of ivermectin in reducing the viral load in mild cases of ambulatory patients showed its advantage over placebo in a double-blind randomized control study [50]. Similar results were obtained in a study in Mexico demonstrating a significant decline on day 5 [51], while other studies did not find any advantage of the drug [52] "The main argument against using ivermectin is that the level of existing evidence for its positive effect is based mainly on studies lacking a high standard of rigorous methodology [53,54]. However, a recent meta-analysis which includes only high-impact journals with low concerns for bias found that ivermectin in comparison to placebo significantly prevents hospitalization (RR 0.77 (CI 0.60-0.98)) [55]. Overall, the use of ivermectin for treating COVID-19 continues to generate significant disagreements worldwide.

The introduction of COVID-19 vaccines was a game-changer in fighting the pandemic. However, it has become clear that relying on vaccines as a sole agent to fight the pandemic is insufficient. The waning immunity phenomenon, which takes place within several months, and the viral mutations which render recently emerging variants insensitive to vaccination, all highlight the need for anti-viral drugs to combat this virus. In fact, there are two new oral anti-SARS-COV-2 drugs which recently received FDA approval and are also authorized for use by health authorities in a number of countries. The first new drug which was manufactured was Molnupiravir (manufactured by Merck) which has only shown a 30% decrease in hospitalization [56]. When compared to the meta-analysis data collected on ivermectin it appears that both drugs render similar results [55]. The second new drug was Nirmatrelvir, which is administrated together with Ritonavir under the brand name Paxlovid (manufactured by Pfizer) and has shown a reduction of 89% in hospitalization albeit in non-vaccinated patients [5]. However, published post-marketing data during the Omicron pandemic and in real-life clinical settings, Paxlovid reduced the risk of hospitalization by only 46% [57]. The principal disadvantage of Paxlovid is its potential and serious interactions with a number of drugs, as well as its contraindication for use in patients with certain medical conditions, who are often the ones who need this drug. Another drawback of these two drugs is the fact that they should be administered within five days of symptom onset, and the treatment course costs several hundred USD. In fact, a recent post-marketing study in Israel has shown that only about 3% of eligible patients did actually take the medication [57]. Thus, the need for other drugs, or combined drug therapy to combat the current

COVID-19 pandemic, is extremely important. Our study, together with other studies concerning the effect of ivermectin, highlights the potential role of this drug in the arsenal of anti-corona drugs. Therefore, even if ivermectin has no clinical benefit as a sole drug, it might have a beneficial effect when combining it to another anti SARS-CoV-2 agent.

Author Contributions:

A.D.S., N. A., and T.M. performed the experiments.

O. E. provided and calibrated the reagent for the RT-qPCR to all coronavirus variants.

M. M., D. A., and E. S. conceived and planned the experiments, analyzed and interpreted the results supervised the project, and led the writing of the manuscript.

All authors have read and agreed to the published version of the manuscript."

Funding: Dakeh Shahin's fellowship was funded by The Meir and Edith Rosenfeld Foundation Institution

Institutional Review Board Statement: All the experiments were done in cells in a culture with viruses that were also grown and isolated from cell culture. First isolation of Viruses were from positive nasopharyngeal swab samples.
Since the virus was isolated from the swab media that was added to the cells in culture, and in practice no human tissue or material was used, according to the **Institutional Review Board** committee patient's consent was not required.
This was approved by the Institutional Review Board, approval number 7875-20-SM

Conflicts of Interest: The authors declare no conflict of interest.

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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

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