



Research Paper

Antiviral effect of high-dose ivermectin in adults with COVID-19: A proof-of-concept randomized trial

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ABSTRACT

Background: There are limited antiviral options for the treatment of patients with COVID-19. Ivermectin (IVM), a macrocyclic lactone with a wide anti-parasitary spectrum, has shown potent activity against SARS-CoV-2 *in vitro*. This study aimed at assessing the antiviral effect of IVM on viral load of respiratory secretions and its relationship with drug concentrations in plasma.

Methods: Proof-of-concept, pilot, randomized, controlled, outcome-assessor blinded trial to evaluate antiviral activity of high-dose IVM in 45 COVID-19 hospitalized patients randomized in a 2:1 ratio to standard of care plus oral IVM at 0.6 mg/kg/day for 5 days versus standard of care in 4 hospitals in Argentina. Eligible patients were adults with RT-PCR confirmed SARS-CoV-2 infection within 5 days of symptoms onset. The primary endpoint was the difference in viral load in respiratory secretions between baseline and day-5, by quantitative RT-PCR. Concentrations of IVM in plasma were measured. Study registered at ClinicalTrials.gov: NCT04381884.

Findings: 45 participants were recruited (30 to IVM and 15 controls) between May 18 and September 9, 2020. There was no difference in viral load reduction between groups but a significant difference was found in patients with higher median plasma IVM levels (72% IQR 59–77) versus untreated controls (42% IQR 31–73) ($p = 0.004$). Mean ivermectin plasma concentration levels correlated with viral decay rate ($r: 0.47, p = 0.02$). Adverse events were similar between groups. No differences in clinical evolution at day-7 and day-30 between groups were observed.

Interpretation: A concentration dependent antiviral activity of oral high-dose IVM was identified at a dosing regimen that was well tolerated. Large trials with clinical endpoints are necessary to determine the clinical utility of IVM in COVID-19.

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Research in context

Evidence before this study

The potential role of ivermectin against SARS-CoV-2 was first reported in April 2020 when an Australian group published *in vitro* results. Since then, multiple opinion papers and a few studies tried to understand the meaning of those results and utility of ivermectin in COVID-19. Mostly observational reports suggest a potential activity that needs confirmation through randomized controlled trials.

Added value of this study

Our study contributes evidence of the antiviral activity of ivermectin against SARS-CoV-2 in patients with COVID-19 through a randomized, controlled, outcome-assessor blinded clinical trial with innovative analyses that include the use of quantitative viral load determinations and measurement of ivermectin plasma levels, which allow an in-depth interpretation of the data and the identification of ivermectin systemic concentrations needed for a significant antiviral effect. The use of an untreated control group highlights the need for controlled trials and on the viral load dynamics in the natural history of COVID-19. Finally, we also add further information on the safety of high dose ivermectin.

Implications of all the available evidence

A concentration dependent antiviral effect of ivermectin in COVID-19 was identified, with significant reductions in SARS-CoV-2 viral load in respiratory secretions among patients achieving high systemic ivermectin concentration compared to untreated controls. These results, that did not show toxicity related to the use of high dose ivermectin, provide evidence of the antiviral effect and support the design of trials to investigate the clinical implications of our findings. Further exploration of the factors involved in the oral bioavailability of ivermectin are also warranted.

1. Introduction

The emergence of a novel coronavirus, Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) in Wuhan in December 2019 and its pandemic spread causing COVID-19 at a global scale, with over 85 million reported cases and 1.7 million deaths by the end of 2020 has prompted the search for pharmacologic interventions to treat, prevent and mitigate the consequences of this potentially devastating acute respiratory infection. Several therapeutic agents have been evaluated at different disease stages as potential antiviral therapies; most of them as part of a drug repurposing strategy for active principles already used in other therapeutic indications. Although different molecules such as hydroxychloroquine, lopinavir and remdesivir have demonstrated antiviral activity against SARS-CoV-2 *in vitro*, evidence from randomized controlled clinical trials has only demonstrated clinical benefits for intravenous remdesivir in some groups of hospitalized patients [1].

Ivermectin (IVM) is a widely used antiparasitic drug with over 900 million tablets distributed in 2019 through the Mectizan Donation Program for the treatment of onchocerciasis and lymphatic filariasis [2]. More recently, several viral infections like Dengue, Zika, and Influenza were shown to be susceptible *in vitro* most likely through host-based mechanisms [3]. A potent activity against SARS-CoV-2 was reported in Vero-hSLAM cell cultures using high concentrations of IVM [4]. In a model of SARS-CoV-2 viral kinetics with acquired immune response to investigate the dynamic impact of timing and dosing regimens, the most significant effects for ivermectin were identified with earlier and longer exposure at high doses; in this regard, repeated daily doses of ivermectin at 600 $\mu\text{g}/\text{kg}$ had meaningful impact whereas doses of 300 $\mu\text{g}/\text{kg}$ had significantly lower effects [5]. Doses of 300 $\mu\text{g}/\text{kg}$ were recently found not to be superior to placebo in a randomized clinical trial in Colombia [6]. IVM is prescribed in weight-based regimens, most frequently at 200 $\mu\text{g}/\text{kg}$, with a proposed link between C_{max} and toxicity [7]. Higher dose regimens are under evaluation due to their potential utility for new indications and dosing strategies [8,9]. Single dose regimens of up to 2000 $\mu\text{g}/\text{kg}$ have been used in a trial in healthy volunteers without clinically significant safety issues [10].

To evaluate the antiviral activity and safety profile of high dose IVM in COVID-19 patients we completed a proof-of-concept randomized controlled clinical trial in hospitalized patients. To achieve further insights into the potential clinical utility of IVM in COVID-19, the relationship between pharmacokinetic (PK) (IVM plasma concentrations) and pharmacodynamic (PD) (dynamic of the viral load) aspects was investigated. Here we present the results of the trial with descriptions on the impact of IVM on SARS-CoV-2 viral load in respiratory secretions.

2. Methods

2.1. Study design

Pilot, multicenter, randomized, open label, outcome assessor blinded, controlled study to assess the antiviral activity and safety of a 5-day regimen of high dose IVM versus no treatment in a 2:1 allocation ratio, in patients with COVID-19. All patients in both groups received standard of care which at that moment in the study area included hospitalization of all symptomatic cases. The trial was done at 4 hospitals in the metropolitan area of Buenos Aires, Argentina.

Ethical approval was obtained from the Institutional Independent Ethics Committees and national regulatory agencies. All participating individuals provided written informed consent. The trial was done in accordance with the principles of the Declaration of Helsinki and is registered with ClinicalTrials.gov, NCT04381884. This study conformed to the CONSORT 2010 guidelines. The funding sources had no role on the design, analysis or decision to publish the results of this study.

2.2. Participants

Participants were COVID-19 patients aged 18 to 69 years-old with RT-PCR confirmed infection, hospitalized and not requiring intensive care. Eligibility criteria included COVID-19 symptoms onset ≤ 5 days at recruitment, absence of drugs with potential activity against SARS-CoV-2 (hydroxychloroquine, lopinavir, remdesivir and azithromycin); and those drugs were not permitted during the first week of

the trial. Exclusion criteria included the use of immunomodulators within 30 days of recruitment, pregnancy, breast feeding and poorly controlled comorbidities. Patients of child-bearing age (men and women) were eligible if agreed to take effective contraceptive measures during the study period and for at least 30 days after the last study drug administration.

2.3. Randomization and masking

A blocked randomization with random block sizes (of 3 or 6 allocations) and stratified by center was used. The randomization list was developed prior to study initiation and by means of a centralized eCRF/IWRS web system (Jazz Clinical, Buenos Aires, Argentina). For reproducibility, a random seed of 1701214029 was used. Once the availability of the informed consent and the verification of all eligibility criteria had been confirmed, the assignment was communicated to the investigators on the computer screen and by email. The patients and center personnel were not blinded to the allocated group. The outcome assessors (personnel in charge of viral load determinations) were blinded to the allocated group upon receiving the samples labeled with the randomization number and the visit number.

2.4. Procedures

All patients were evaluated at study entry with full history and physical exam. Patients in the IVM group received oral treatment for 5 consecutive days with either breakfast or lunch at approximately 24 h intervals. IVM 6 mg ranurated tablets (IVER P, Laboratorios Elea/Phoenix, Argentina) were used in all cases at a dose of 600 $\mu\text{g}/\text{kg}/\text{day}$ based on baseline weight rounding to the lower full (6 mg) and half (3 mg) dose. The regimen of 600 $\mu\text{g}/\text{kg}$ for 5 days was selected based on the *in-vitro* data suggesting the need for higher doses than for current indications of IVM, the available data on the safety of this dose in regimens of up to 3 days (either in fast or fed state) and the available information on the PK of IVM, predicting the lack of significant accumulation of IVM after 5 daily doses [8,9,11,12]. Nasopharyngeal swabs were collected at baseline and 24, 48 and 72 h and on day 5 for SARS-CoV-2 viral load quantification. Blood samples were obtained by venipuncture for plasma IVM concentrations 4 h after drug intake on treatment days 1, 2, 3, and 5 (aiming at measuring peak plasma levels) and on day 7 (aiming to evaluate potential drug accumulation) in the IVM group. Blood samples were obtained from participants in both groups for hematologic and chemical parameters.

2.5. Outcomes

The primary outcome measure was the difference in SARS-CoV-2 viral load between baseline and day-5 in both groups. Secondary outcomes included clinical evolution at days 7 and 30, relationship between IVM plasma concentrations and the primary outcome, and frequency and severity of adverse events.

2.6. SARS-CoV-2 viral load measurements

Viral load of SARS-CoV-2 from nasopharyngeal swabs was quantified from samples stored at -80°C until use. Viral RNA was extracted using QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) from 140 μL of stored samples. Then, an in-house reverse transcriptase quantitative PCR (RT-qPCR) targeting *N* gene of SARS-CoV-2 was performed. The standard curve consisted of an *in vitro* transcribed viral RNA serially diluted in a cellular RNA matrix from negative nasopharyngeal samples. This assay included the measure of a housekeeping gene as an internal control and normalizer. The housekeeping gene cycle threshold (Ct) was used to correct the specific-SARS-CoV-2 Ct according to the number of cells in the sample. Therefore, viral load

measurements were expressed as \log_{10} copies per reaction instead of \log_{10} copies per mL as discussed by Han and colleagues [13]. The performance of the assay includes: (i) efficiency = 99%, (ii) reproducibility with a coefficient of variation (CV) between 1.01 and 2.31, (iii) repeatability with a CV between 0.27 and 1.89%, (iv) dynamic range from 10 to 1×10^8 copy per reaction, (v) specificity = 100% tested against SARS-CoV-2 negative samples and a panel of respiratory viruses. All these parameters were determined according to the guidelines for *in vitro* quantitative diagnostic assays as were reported previously [14,15].

2.7. Measurement of IVM plasma concentration profiles

IVM concentrations in plasma samples were determined by High-Performance Liquid Chromatography (HPLC) with fluorescence detection. The chromatography technique was adapted as previously described [16]. An aliquot of plasma was combined with moxidectin (used as internal standard). After an acetonitrile-mediated chemical extraction, IVM was converted into a fluorescent molecule using *N*-methylimidazole and trifluoroacetic anhydride (Sigma Chemical, St Louis, MO, USA). An aliquot (100 μL) of this solution was injected directly into the HPLC system (Shimadzu Corporation, Kyoto, Japan) and analyzed using a reverse phase C18 column (Kromasil, Eka Chemicals, Bohus, Sweden, 5 μm , 4.6 mm \times 250 mm) and an acetic acid 0.2% in water/methanol/acetonitrile (1.6/60/38.4) mobile phase at a flow rate of 1.5 mL/min at 30 $^\circ\text{C}$. Fluorescent detector was set at 365 nm (excitation) and 475 nm (emission wavelength). The coefficient of determination (r^2) of the calibration curve was 0.995. The mean absolute drug recovery percentage was 94%. The precision of the method showed a coefficient of variation below 8.1%. The limit of drug quantitation was 0.3 ng/mL. Drug concentrations in experimental plasma samples were obtained by peak area integration using the Solution Software (Shimadzu Corporation, Kyoto, Japan).

2.8. Pharmacokinetic and pharmacodynamic analysis of the data

IVM plasma concentrations were measured in each patient 4 h post-dosing on the established treatment days. Individual plasma vs time curves were plotted. The pharmacokinetic parameters were determined using PK Solutions 2.0 (Ashland, Ohio, US) computer software. The viral decay rate was calculated from the viral load vs time curve. Following an exponential model, the decay rate constant was calculated from the following equation:

$$\lambda = S \times 2.303$$

where λ is the decay rate constant and S is the slope [17].

2.9. Statistical analysis

Sample size calculation was determined on current recommendations for pilot trials, indicating that either at least 10 cases per group should be included or based on the sample size calculation for the full-scale clinical trial and include at least 9% of that size for a confidence interval of 80% [18,19]. Based on these grounds and aiming for a sample size with the ability to detect a low effect size (0.3) of the intervention (IVM) in the difference between baseline and day-5 viral load values compared to untreated controls given the absence of preliminary or historical data; sample size for a full-scale trial for two study groups with a significance level of 5% and 80% power, a 2:1 randomization and inflated for 10% lost-to-follow-up was calculated in 342 participants and a pilot trial would be at least 31 [19]. In view of the presumed effect of IVM on the replication of SARS-CoV-2 and the limited available information of viral dynamics at the time of study design (April 2020), the sample size of the pilot trial according to standardized size effects [20], was calculated for a 2:1 randomization

to be 45 patients, including 30 participants in the IVM arm and 15 controls without consideration to the center-based stratification.

Baseline characteristics of the two groups (control and ivermectin) were compared with Student's *T*-test and Chi square. Difference in viral load between baseline and day-5 in the two groups as well as the comparison between the viral decay rate of both groups was compared by the non-parametric Mann–Whitney test. The clinical evolution at day-7 was evaluated by Fisher's Exact Test. Finally, the relationship between IVM plasma concentrations with viral load reduction and viral decay rate were measured by Spearman rank test. When difference across three groups by Kruskal–Wallis was significant, pairwise comparisons with Dunn's multiple comparisons test were used. Two randomly occurring single missed values of viral load in two different participants were assumed as "missing completely at random" type of values and estimated by regression analysis using the interpolation of all the existing data from that particular curve. In all cases, *p*-values <0.05 were considered statistically significant. All analysis were performed with GraphPad Prism version 5.00 for Windows (La Jolla California USA).

2.10. Role of the funding source

The sponsors of the study participated in study design, but had no role in primary data collection, data analysis, data interpretation, writing of the report, or the decision to submit for publication. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3. Results

Enrolment started on May 18 and finished on September 9 2020, with 45 participants recruited among 4 participating hospitals. As planned, 30 were randomized to the IVM group and 15 to the untreated control group. Two subjects withdrew consent in the IVM group; in 1 case due to a mild rash and nausea after 1 dose of IVM and the other due anxiety after 2 doses; in both cases, adverse events resolved spontaneously; the remaining 28 subjects in the IVM group completed treatment. One case in the control group was withdrawn from the study due to the initiation of lopinavir on day-5 due to disease progression and another was lost to follow-up after the visit on day-7. In addition, ten cases, seven in the IVM group and three controls, presented viral load below the limit of quantification (<10 copies/reaction) at baseline. All these cases remaining undetectable in most samples through the follow-up and excluded from the efficacy analysis. The remaining 32 cases (20 treated and 12 control) constitute the efficacy analysis population (Fig. 1).

Baseline characteristics are summarized in Table 1. Comorbidities and disease stages were similar between groups with the most frequent comorbidity being a higher-than-normal body mass index (spanning from overweight to obesity grade III) in both groups, which was present in 19 (63%) in the IVM group and 12 (80%) controls (*p* = 0.43) (Table 1). No differences in clinical symptoms, signs, or laboratory parameters were observed between groups at baseline and the two groups did not show differences in the number of individuals on the WHO-ordinal scale categories. Disease progression was registered in 3 (7%) of the study population; 2 in the treated group and 1 in among the controls, with 1 case in the IVM group requiring invasive mechanical ventilation and no significant differences in clinical evolution at day-7 and day-30 between groups. No deaths occurred through the study period.

The difference in viral load between baseline and day-5 was similar between groups and decreasing over time, without significant differences (Fig. 2). No differences in baseline viral load were detected between males and females. Viral load values under the limit of quantification of 10 copies/reaction at day-5 were achieved by 6 of 20 (30%) subjects in the IVM group and in 1 of

12 (8.3%) in the control group without statistical significance between groups. When mean plasma IVM concentration levels were analyzed in relation to reduction in viral load, a significant positive correlation was identified, with those patients achieving higher mean plasma concentrations of IVM reaching higher reductions in viral load in nasopharyngeal secretions (*r*: 0.44; *p* < 0.04). Mean IVM plasma concentration levels also showed a positive correlation with viral decay rate (*r*:0.47, *p* = 0.02).

3.1. Data are mean (SD). Day-1 indicates baseline measurements

Based on the observed antiviral response and the observed IVM concentration levels in this study, treated patients were divided in two subgroups with 160 ng/ml as the cutoff plasma concentration for a post-hoc analysis, since it was identified as the potential threshold above which a significant viral load reduction could be established compared to untreated controls as an indicator of the relationship between ivermectin concentrations in plasma and antiviral activity. Median *C*_{max} was 202 ng/ml (IQR: 167–268 ng/ml) in the >160 ng/ml subgroup and 109 ng/ml (IQR: 91–141 ng/ml) in the <160 ng/ml subgroup (*p* < 0.0001). To further explore this PK/PD relationship, viral load dynamics and difference between baseline and day-5 were analyzed in the 2 subgroups of IVM treated patients, with median (IQR) reductions in viral load of 42% (31–73) in the control group, 40% (21–46) in treated patients with <160 ng/ml median plasma concentrations, and 72% (59–77) in the higher concentration group, with a statistically significant difference between the latter and the other groups (Kruskal–Wallis *p* = 0.0096) (Fig. 3). The proportion of subjects achieving viral load values under the limit of quantification at day-5 was 8.3% (1 of 12) in the control group, 9.1% (1 of 11) in the <160 ng/ml subgroup and 55.6% (5 of 9) in the >160 ng/ml subgroup (*p* < 0.0001).

All treated patients receiving IVM 0.6 mg/kg/day for 5 days.

Drug-induced effects on viral clearance were also assessed using viral decay rates as an endpoint parameter and its relationship with IVM plasma concentrations. The viral decay rate in treated patients with IVM plasma levels >160 ng/ml was significantly greater (median 0.64 *d*⁻¹) compared to untreated controls (median 0.13 *d*⁻¹) and to the subgroup with <160 ng/ml median plasma concentrations (median 0.14 *d*⁻¹) (*p* = 0.04) (Fig. 4a). No statistically significant differences in baseline viral load were observed between IVM concentration subgroups. The IVM concentration profiles did not correlate with body weight (*r*:0.1; *p* > 0.05) or body mass index (*r*:0.07; *p* > 0.05) among the 28 patients that completed treatment with IVM.

Adverse events were reported in 18 (40%) of the 45 patients, 13 (43%) in the IVM group and 5 (33%) in the control group (Table 2). The most frequent adverse event and the only experienced by more than 1 case in the IVM group was rash in 3 (10%) cases (all mild, self-limited and lasting approximately 24 h); in the control group, single events of abdominal pain, dizziness, anxiety, anguish, and hyperglycemia (all mild) were reported. A single serious adverse event (SAE) occurred in a patient in the IVM group with hyponatremia, which has been recently recognized in case series of COVID-19 cases and has not been reported in association to IVM use [21].

4. Discussion

This proof-of-concept trial designed to evaluate the antiviral activity of IVM against SARS-CoV-2 in adult patients with COVID-19 showed no differences between treatment and control groups in its primary endpoint which was the difference in SARS-CoV-2 viral load between baseline and day-5 (Fig. 1). However, our results indicate a concentration-dependent antiviral activity of IVM in SARS-CoV-2 infected patients treated within 5 days of symptoms onset. This statistically significant difference was identified for the relationship

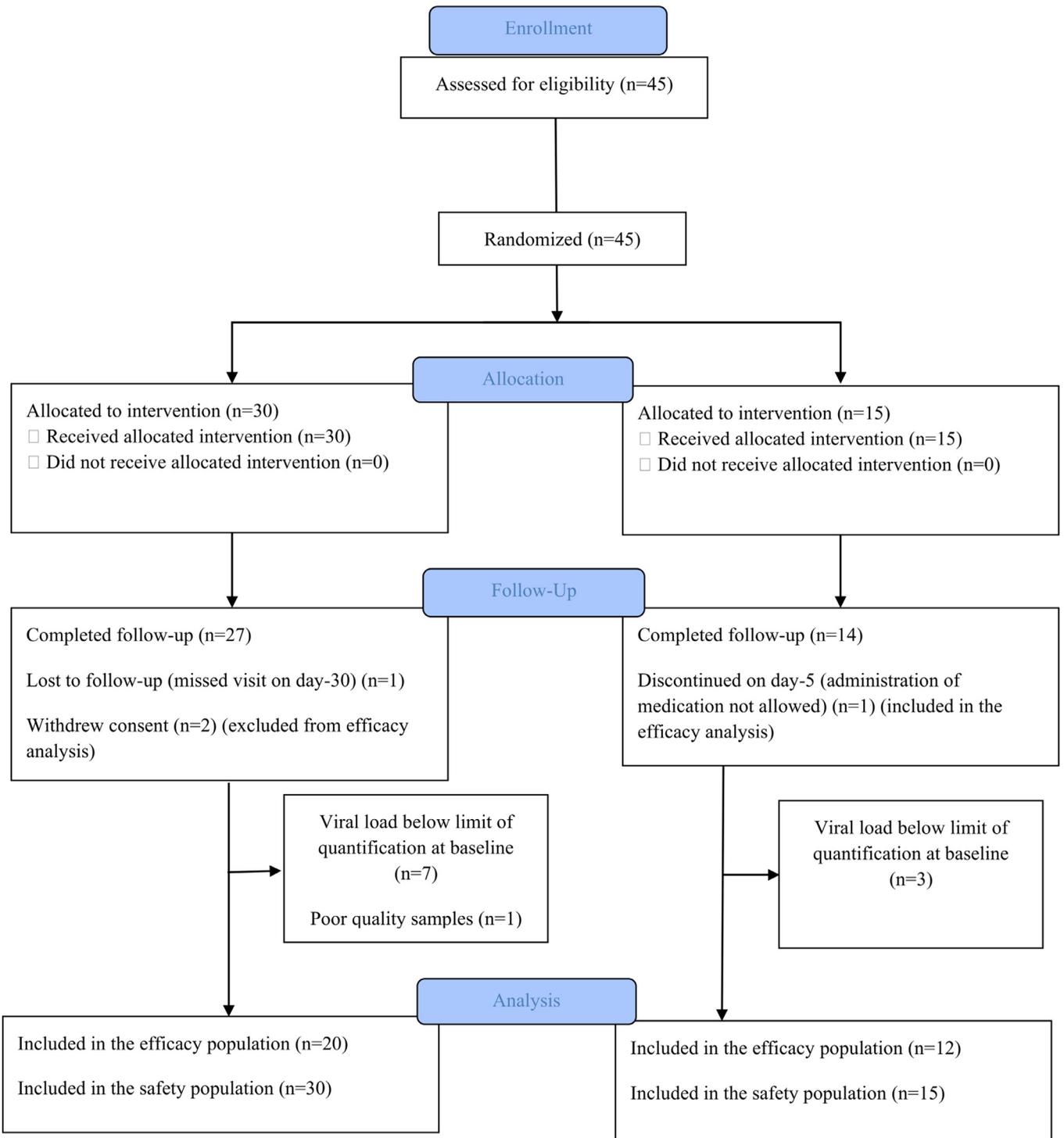


Fig. 1. Trial profile.

between IVM plasma concentrations and the primary outcome (Figs. 3 & 4); which confirms previous *in vitro* activity shown in cell cultures [4]. Findings on IVM plasma concentrations are in agreement with human SARS-CoV-2 viral kinetic models identifying the need for high doses, but contradict those concerns stating that those drug concentrations would not be achievable at safe doses [5,22]. The extensive pattern of IVM distribution to lung tissue has been well characterized in cattle and pigs, with the later also achieving in nasopharyngeal tissue higher levels than plasma [16,23]. Considering that similar volumes of distribution have been reported for IVM in both cattle and humans and the systemic availability observed in this

clinical trial, it is reasonable to estimate median IVM levels >395 ng/g in lung tissue. A similar pattern of IVM distribution to lung tissue has been recently simulated using a minimal physiologically based PK model [24].

The antiviral effect was seen after IVM plasma concentration measurements allowed discrimination between patients achieving higher levels and identifying a direct relationship between drug concentration and viral elimination. Additionally, relevant conclusions on the natural history of the illness can be derived from the behavior of the control group in this trial, which demonstrates the self-limited nature of viral load in SARS-CoV-2 infections, that in 22% of the cases

Table 1
Baseline characteristic of the study population.

	Control (n = 15)	Ivermectin (n = 30)	P value
Age (year)	38•1 ± 11•7	42•3 ± 12•8	0•29
Gender			
Female	5 (33%)	15 (50%)	0•29
Male	10 (67%)	15 (50%)	
Weight (kg)	79•7 ± 14•4	75•3 ± 15•0	0•35
Overweight	8 (53%)	6 (20%)	0•05
Obesity I	2 (13%)	11 (37%)	0•20
Obesity II	1 (7%)	1 (3%)	0•79
Obesity III	1 (7%)	1 (3%)	0•79
Oxygen saturation <94%	0	1 (3%)	0•63
Log viral load (log10 copies/reaction)	5•39 ± 1•56 (n = 12)	4•18 ± 1•60 (n = 20)	0•05
Hematology			
White blood cell count (cell/ μ L)	4857 ± 1874	6014 ± 2402	0•09
Lymphocyte count (cell/ μ L)	1478 ± 266	1744 ± 747	0•09
Biomarkers			
Lactate dehydrogenase (IU/L)	460 ± 117	468 ± 140	0•85
Ferritin (mg/dL)	1318 ± 1969	1071 ± 1304	0•66
D-dimer (μ g/mL)	1•5 (0•1–2•8)	1•5 (0•5–1•8)	0•82
Time from symptoms onset (day)	3•6 ± 1•4	3•5 ± 1•0	0•78
Body temperature $\geq 37\cdot5$ °C	1 (7%)	4 (13%)	0•70
WHO-ordinal scale			
3	13 (87%)	29 (97%)	0•20
4	2 (13%)	1 (3%)	
Ground glass opacities in thoracic imaging	6 (40%)	14 (47%)	0•67
Comorbidities			
Hypertension	3 (20%)	3 (10%)	0•35
Diabetes	1 (7%)	6 (20%)	0•24
Chronic lung disease/Asthma	1 (7%)	4 (13%)	0•50

Numeric variables are reported as median (IQR) or mean \pm standard deviation • Categorical variables are reported as counts (%). Overweight: Body mass index (BMI) 25–29•9 kg/m²; Obesity I: BMI 30–34•9 kg/m²; Obesity II: BMI 35–39•9 kg/m²; Obesity III: BMI >40 kg/m².

was already below the limit of quantification at baseline; a finding similar to what has been observed in a trial evaluating remdesivir for the treatment of COVID-19 [25], highlighting the relevance of adequate timing of implementation of antiviral treatment as has been shown in a recently published pilot double-blind trial randomized trial of IVM for non-severe COVID-19 that identified statistically

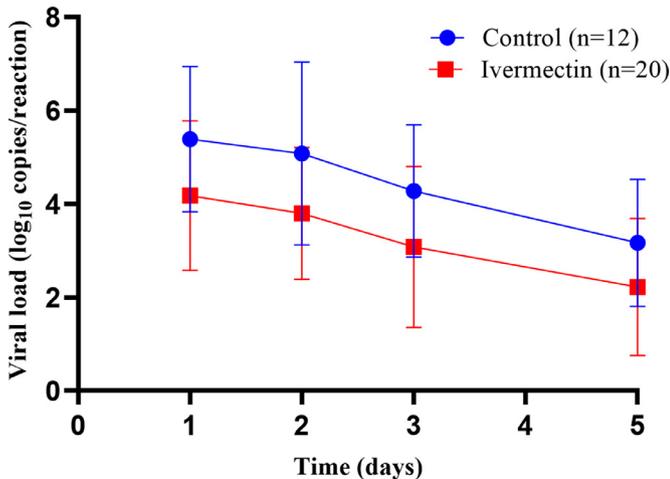


Fig. 2. Viral load by quantitative RT-PCR on upper respiratory tract secretions since baseline in patients receiving IVM 0•6 mg/kg/day for 5 days versus untreated controls.

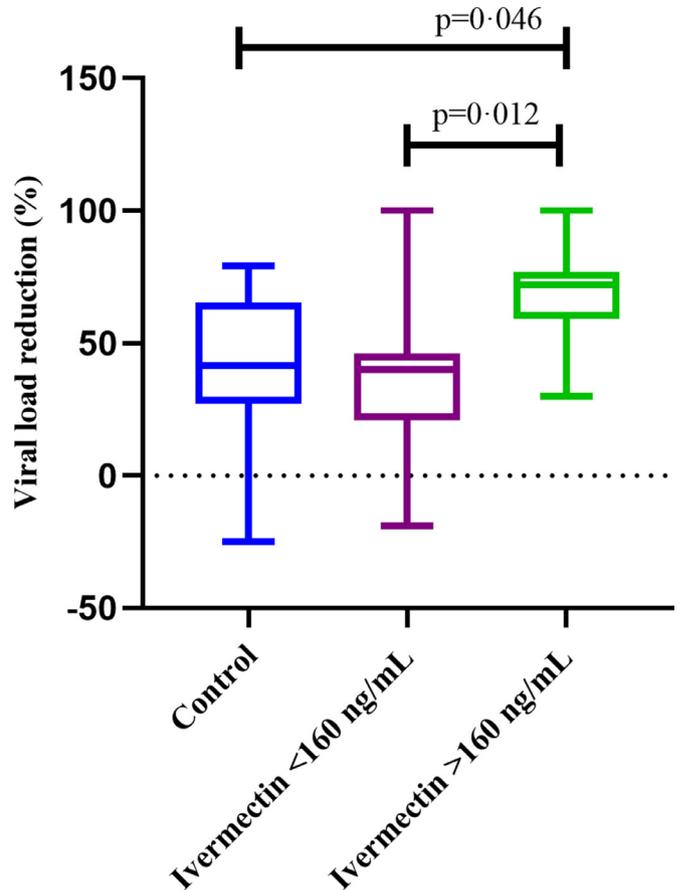


Fig. 3. Viral load reduction between baseline and day-5 (median and IQR) in untreated controls and IVM treated patients discriminated by their median IVM plasma concentrations.

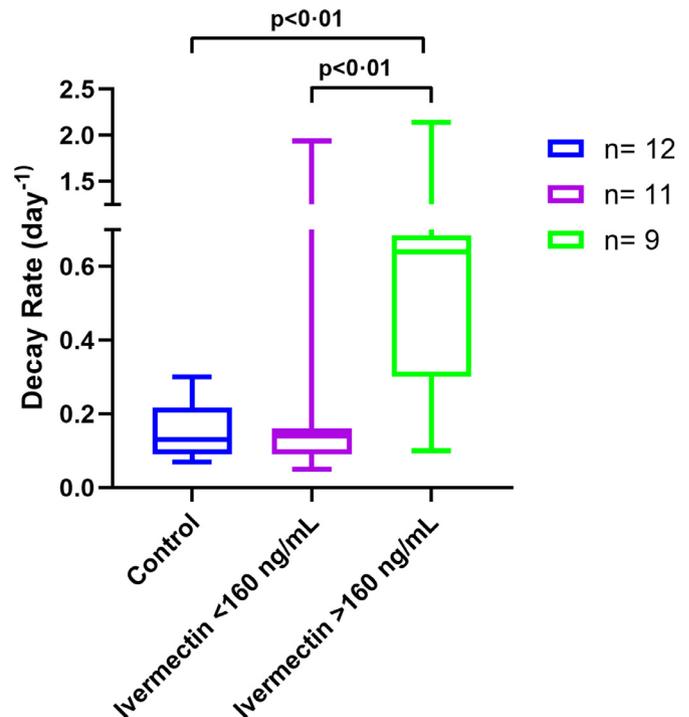


Fig. 4. Viral load decay rates by quantitative RT-PCR on upper respiratory tract secretions in untreated controls and IVM treated patients according to median plasma concentrations of IVM. Data are expressed as median (IQR).

Table 2
Summary of events in safety population.

	Control (n = 15)	Ivermectin (n = 30)
Patients with AEs	5 (33%)	13 (43%)
Patients with possible/probable related AEs	NA	9
Patients with SAEs	0	1*
Patients with possible/probable related SAEs	0	1
Number of AEs	5	17
Number of possible/probable related AEs	NA	11
Number of AEs Grade 3/4	0	3**

AE: adverse event. SAE: serious adverse event. *: hyponatremia; **: includes the SAE (hyponatremia) and ALT and AST elevation, both in the same patient.

significant differences in the duration of anosmia and trends towards lower viral load with treatments started within 72 h of symptoms onset [26].

IVM plasma concentrations >160 ng/mL were measured in 9 (45%) patients included in the efficacy analysis population. In a trial using a 3-day regimen of 600 µg/kg for malaria control among adults, median C_{max} (CI95%) was 119 ng/mL (45–455) [8]. Diet is a key variable affecting oral bioavailability of IVM, with increased plasma concentrations achieved with fed state [27,10]. The interaction of IVM with ABC transporters as P-glycoprotein and the modulation of P-glycoprotein activity after oral administration is well known [28,29]. Thus, variable constitutive and/or induced level of expression and activity of intestinal P-glycoprotein in treated patients, may have contributed to the observed large variability in the pattern of IVM absorption and systemic exposure.

Although further information is needed, this pilot trial adds evidence on the safety of multiple-day high-dose regimens of IVM, without unexpected findings; in agreement with a trial in Kenya evaluating the mosquitocidal effect of IVM at 300 and 600 µg/kg in adults with malaria [9]. In that trial, IVM was associated with dose-dependent mild transient visual disturbances in <10% of the participants which were not reported in the current study [9]. The frequency of adverse events reported by study participants (43% of those in the IVM group and 33% of the untreated controls) (Table 2), likely reflects events related and unrelated to the study drug which as expected were more frequent in the treated groups, although most of them of severity grades 1 and 2.

Limitations of this study include its sample size, which is based on demonstrating the antiviral activity of IVM against SARS-CoV-2 but lacks power to detect differences in clinical outcomes. The analysis of the primary outcome based on days since study entry rather than since symptom onset might have added a source of variability in viral load values and curves, which was partially controlled by using the difference between baseline and day-5 rather than the longitudinal trajectory of the curves; and although both treatment arms were balanced in terms of comorbidities and disease severity (Table 1), no adjustments regarding infection stage or comorbidities were made in the analysis, which might constitute a minor limitation of this study. The lack of a registry of the meals ingested around the intake of each treatment may add a source of variation to the observed IVM plasma profiles. The utilization of a highly sensitive viral load measurement method with the ability to reliably quantify as low as 10 copies/reaction might have caused an underestimation of the antiviral activity of IVM in its capacity to lower viral load values below the lowest level of quantification. Although not achieving statistically significant differences between groups, the wide dispersion of baseline viral load and the baseline difference in viral loads between groups are limitation of this study. Two viral load values among the 128 individual viral load measurements used for the primary outcome, were “missed completely at random” type of values due to technical

reasons and estimated using regression analysis; those two values constitute a minor limitation of this study.

The assessment of the effect of drug candidates against viruses causing acute respiratory infections is hampered by several aspects of these host-pathogen relationships including rapid immune control of viral replication and high variability in symptom scores among patients [30]. For that reason, key components for adequate endpoints are sensitive quantifiable measurements of the underlying cause as the quantitative RT-PCR [31]. As it has been proposed in an influenza model of antiviral candidate drugs evaluation²⁵, viral decay rates proved to be a critical parameter of antiviral activity. Additionally, as it has been clearly demonstrated for acute viral infections, early treatment initiation plays a critical role [31,32]. The clinical relevance of these findings remains to be confirmed in trials with clinical endpoints. Beyond clinical aspects, lowering viral burden might influence infectivity, although there is conflicting data regarding the relationship between burden of viral shedding and infectivity [33]. The proposed antiviral mechanism of IVM is through its ability to inhibit the nuclear import of viral proteins mediated by IMPα/β1 heterodimer [4], and it has also been suggested that IVM could promote defense mechanisms such as pyroptosis in infected epithelial cells [34]. Drugs such as ivermectin can be used to target viral entry or viral replication mechanisms in host cells, as well as to modulate the innate immune responses, to achieve indirect antiviral activity *in vivo*. Mechanisms essential for viral infection, such as nuclear transport or intracellular signal transduction, among others, have been indicated as better targets to identify broad-spectrum antiviral agents, with some advantages over direct-acting antivirals targeting viral components [35].

In summary, our findings support the hypothesis that IVM has a concentration dependent antiviral activity against SARS-CoV-2 and provides insights into the type of evaluations to be considered in the assessment of antiviral drugs for the control of COVID-19. Follow-up trials to confirm our findings and to identify the clinical utility of IVM in COVID-19 are warranted.

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Declaration of Competing Interest

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Data sharing

De-identified individual clinical and laboratory data and a data dictionary will be made available to others after 3 months of trial publication upon request to the corresponding authors, only for research, non-commercial purposes to individuals affiliated with academic or public health institutions.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi: [10.1016/j.eclinm.2021.100959](https://doi.org/10.1016/j.eclinm.2021.100959).

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