

Antiviral effect of high-dose ivermectin in adults with COVID-19: a pilot randomised, controlled, open label, multicentre trial.

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Summary

Background

There are limited antiviral options for the treatment of patients with coronavirus disease 2019 (COVID-19) that have demonstrated clinical efficacy and none of them is an oral drug. Ivermectin (IVM), a macrocyclic lactone with a wide anti-parasitary spectrum, has shown potent in vitro activity against SARS-CoV-2 in cell cultures.

Methods

We completed a pilot, randomized, controlled, outcome-assessor blinded clinical trial with the goal of evaluating the antiviral activity of high dose IVM in COVID-19 patients. Eligible patients were adults (aged 18 to 69 years) with mild or moderate RT-PCR confirmed SARS-CoV-2 infection within 5 days of symptoms onset. 45 patients were 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. The primary endpoint was viral load reduction in respiratory secretions at day-5. Viral load in respiratory secretions was measured through quantitative RT-PCR. Concentrations of IVM in plasma were measured on multiple treatment days. This trial is registered with ClinicalTrials.gov, NCT004381884.

Findings

The trial run between May 18 and September 29, 2020 with 45 randomized patients (30 in the IVM group and 15 controls). There was no difference in viral load reduction between groups but a significant difference in reduction was found in patients with higher median plasma IVM levels (72% IQR 59 – 77) versus untreated controls (42% IQR 31 – 73) ($p=0.004$). The mean ivermectin plasma concentration levels also showed a positive correlation with viral decay rate ($r:0.47$, $p=0.02$). Adverse events were reported in 5 (33%) patients in the controls and 13 (43%) in the IVM treated group, without a relationship between IVM plasma levels and adverse events.

Interpretation

A concentration dependent antiviral activity of oral high dose IVM was identified in this pilot trial 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 uncontrolled studies tried to understand the meaning of those results and utility of ivermectin in COVID-19. We searched clinicaltrials.gov on October 16 and identified 39 registered trials. A Pubmed search on the same date identified 72 published articles on (“COVID” or “SARS”) AND “ivermectin”, none of them a clinical trial or randomized controlled trial.

Added value of this study

Our study is the first contribution that provides evidence of the antiviral activity of ivermectin against SARS-CoV-2 in patients with COVID-19 through a randomised, controlled, outcome-assessor blinded clinical trial. The incorporation of quantitative viral load determinations and measurements of ivermectin plasma levels allows 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.

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 33 million reported cases and 1 million deaths by the end of September 2020 has prompted the search of 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 sub-groups of hospitalized patients. ¹

Ivermectin (IVM) is a widely used antiparasitic drug against several filarial diseases, scabies, and strongyloidiasis, with over 900 million tablets distributed in 2019 through the World Health Organization (WHO) for the treatment of onchocerciasis and lymphatic filariasis as part of WHO's Essential Medicines List. ^{2,3} After decades of intensive, safe, and effective use, IVM has more recently been evaluated for potential new indications including malaria and several viral infections that were shown to be susceptible to IVM *in vitro* like Dengue, Zika, and Influenza. ⁴ A potent activity against SARS-CoV-2 was reported in Vero-hSLAM cells cultures using high concentrations of IVM.⁵ Different pharmacokinetic (PK) and pharmacodynamic (PD) models suggested that the extremely high plasma concentrations necessary for the antiviral effect observed *in vitro* would require doses far from those safely used in humans, in order to reach concentrations close to 2 μM , the half-maximal inhibitory concentration (IC₅₀) required against SARS-CoV-2 *in vitro*. ⁶ IVM is prescribed in weight-based regimens, most frequently at 0.2 mg/kg, although 0.4 mg/kg is approved for *Wuchereria bancrofti* infections.⁷ Based on its safety profile, higher dosing regimens are under evaluation due to their potential utility for new indications and dosing strategies. ^{7,8}

To evaluate the antiviral activity and safety profile of high dose IVM in COVID-19 patients and to advance the knowledge on the role of this drug, we completed a proof of concept randomized controlled clinical trial in hospitalized patients with mild and moderate disease. To achieve further insights into the potential clinical utility of IVM in COVID-19, the relationship between drug PK (systemic exposure) and 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.

Methods

Study design and participants

A multicenter, individually randomized, open label, outcome assessor blinded, controlled clinical trial 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 adult hospitalized patients with mild to moderate COVID-19. All patients in both groups received standard of care. 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 from district 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. This study is registered with ClinicalTrials.gov, NCT004381884.

Eligible participants were COVID-19 patients aged 18 to 69 years-old with RT-PCR confirmed infection, hospitalized with disease stages 3 to 5 from the WHO 8-Category ordinal scale of clinical status and no requiring intensive care unit admission. Eligibility criteria included COVID-19 symptoms onset ≤ 5 days at recruitment, absence of use of drugs with potential activity against SARS-CoV-2 and available in Argentina during the trial (hydroxychloroquine, chloroquine, lopinavir 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, poorly controlled comorbidities and known allergies to IVM. Patients of child-bearing age (men and women) were eligible if agreed to take effective contraceptive measures (including hormonal contraception, barrier methods, or abstinence) during the study period and for at least 30 days after the last study drug administration.

Randomization and masking

Enrolled participants were randomly assigned (2:1) to either IVM group or untreated control group. Randomization was stratified for each Center. Randomization sequence was prepared by a centralized, web-based system in blocks of variable size (3, 6 or 9 cases per block) and communicated to the trial physicians that recruited the patients upon entry to the web system information on availability of the signed Informed Consent Form and verification of all eligibility criteria. Patients, nurses, and physicians were not blinded to the treatment arm. Outcome assessors (virology staff) were blinded to the treatment group by receiving the samples labeled with randomization code and visit number.

Procedures

All participating patients were evaluated at study entry when a full history and physical exam were performed, including weight and height. Patients in the IVM group received oral treatment for 5 consecutive days with either breakfast or lunch at approximately 24 hours intervals. IVM 6 mg ranurated tablets (IVER P, Laboratorios Elea/Phoenix, Argentina) were used in all cases at a dose of 0.6mg/kg/day based on baseline weight rounding to the lower full (6mg) and half (3mg) dose. Patients were assessed daily by hospital nurses and physicians from the research teams in each hospital during the 7-day initial period and at day 21 to 30 from study entry. All clinical findings, including adverse events and concomitant medication, were entered in paper based clinical records and then entered into an electronic case report form that was monitored and validated by trial staff. Nasopharyngeal swabs were collected at baseline and 24, 48 and 72 hours and on day 5 for SARS-CoV-2 viral load quantification. Blood samples were obtained by venipuncture for plasma IVM concentrations, 4 hours after drug intake on treatment days 1, 2, 3, and 5 (aiming at measuring peak plasma levels) and on day 7 (aiming to estimate drug elimination) in the IVM group. Blood samples were obtained from participants in both groups for hematologic and chemical parameters.

Outcomes

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

SARS-CoV-2 viral load measurements

Viral load of SARS-CoV-2 from nasopharyngeal swabs was quantified from samples stored in viral transport medium at -80°C until use. For the viral load assay, 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 log₁₀ copies per reaction instead of log₁₀ copies per mL as discussed by Han and colleagues.⁹ The performance of the assay includes: i) efficiency = 99%, ii) reproducibility with a coefficient of variation (CV) between 1.01 to 2.31, iii) repeatability with a CV between 0.27 to 1.89%, iv) dynamic range from 10 to 1 x 10⁸ 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.^{10,11}

Measurement of IVM plasma concentration profiles

Concentrations of IVM in plasma samples collected from treated patients were determined by High-Performance Liquid Chromatography (HPLC) with fluorescence detection. The chromatography technique was adapted as previously described.¹² 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). HPLC analysis was undertaken using a reverse phase C18 column (Kromasil, Eka Chemicals, Bohus, Sweden, 5 µm, 4.6 mm × 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 °C. Fluorescent detector was set at 365 nm (excitation) and 475 nm (emission wavelength). Full validation of the analytical procedures used to measure IVM plasma concentrations was performed. The determination coefficient (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).

Pharmacokinetic and pharmacodynamic analysis of the data

The drug plasma concentrations measured in each patient at the different post-treatment times were plotted. The pharmacokinetic parameters were determined using PK Solutions 2.0 (Ashland, Ohio, US) computer software. The area under the IVM concentration-time curves for (AUC_{ivm}) (named as systemic drug exposure) were calculated by the trapezoidal rule.¹³ The estimation of the area under the viral load-time curves (AUC_{vl}) was estimated as a pharmacodynamic indicator of drug activity. The viral decay rate was calculated from the viral load curve-time using 4 points as $2.303 \times \text{slope}$.¹³ The data analysis included the estimation of the drug PK/PD relationship. The ratio between drug exposure (PK) (expressed as AUC_{ivm}) and the dynamic of the viral load (PD) (expressed as AUC_{vl}) was estimated as an indicator of the relationship between drug exposure and antiviral activity.

Statistical analysis

Trial design included a sample size calculation that was determined on current recommendations for pilot trials, indicating at least 10 cases per group¹⁴ 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% (Cocks).¹⁵ Based on these grounds and low effect size (0.3) the sample size for a full-scale trial would be 342 and a pilot of at least 31 based on Cocks *et al.* 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 with an α error of 0.05 a power of 0.80 and a low effect size according to standardized size effects,¹⁶ was calculated for a 2:1 randomization in 30 patients in the IVM group and 15 in the control group, to detect differences between 2 independent groups in the decrease in mean viral load in nasopharyngeal swabs between baseline and day-5.

Groups were compared and the continuous variables were analyzed for statistical significance with the Mann-Whitney and the Kruskal-Wallis non-parametric test. Correlations were analyzed using the Spearman rank test. Data analysis was performed with GraphPad Prism version 5.00 for Windows (La Jolla California USA (www.graphpad.com)). The statistical analysis for PK parameters and percentages of viral load reduction at day 5 post-treatment was performed using the Instat 3.0 software (GraphPad Software, CA, US). Parametric (ANOVA, Bonferroni multiple comparisons) and non-parametric (Kruskal–Wallis) tests were used for the statistical comparison. When difference across three groups was significant, pairwise comparisons were made with Dunn's multiple comparisons test. Non-parametric correlation was used for the relationship between viral load reduction and IVM systemic concentration. In all cases, p-values <0.05 were considered statistically significant. Two missed values of viral load were estimated by regression analysis using the existing data.

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.

Results

Enrolment started on May 18 and finished on September 9, 2020, with 45 participants recruited among the 4 participating hospitals. On September 29, the last scheduled visits were completed. 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; in both cases, the adverse events were judged as possibly related to IVM and resolved spontaneously; the remaining 28 subjects in the IVM group completed treatment. All doses of IVM were completed during hospitalization. One case in the control group was withdrawn from the study due to the initiation of lopinavir on day-5 due to disease progression. All patients assigned to the treatment group started treatment, therefore all subjects were included in the safety analysis (figure 1). One patient in the control group was lost to follow-up after the visit on day-7.

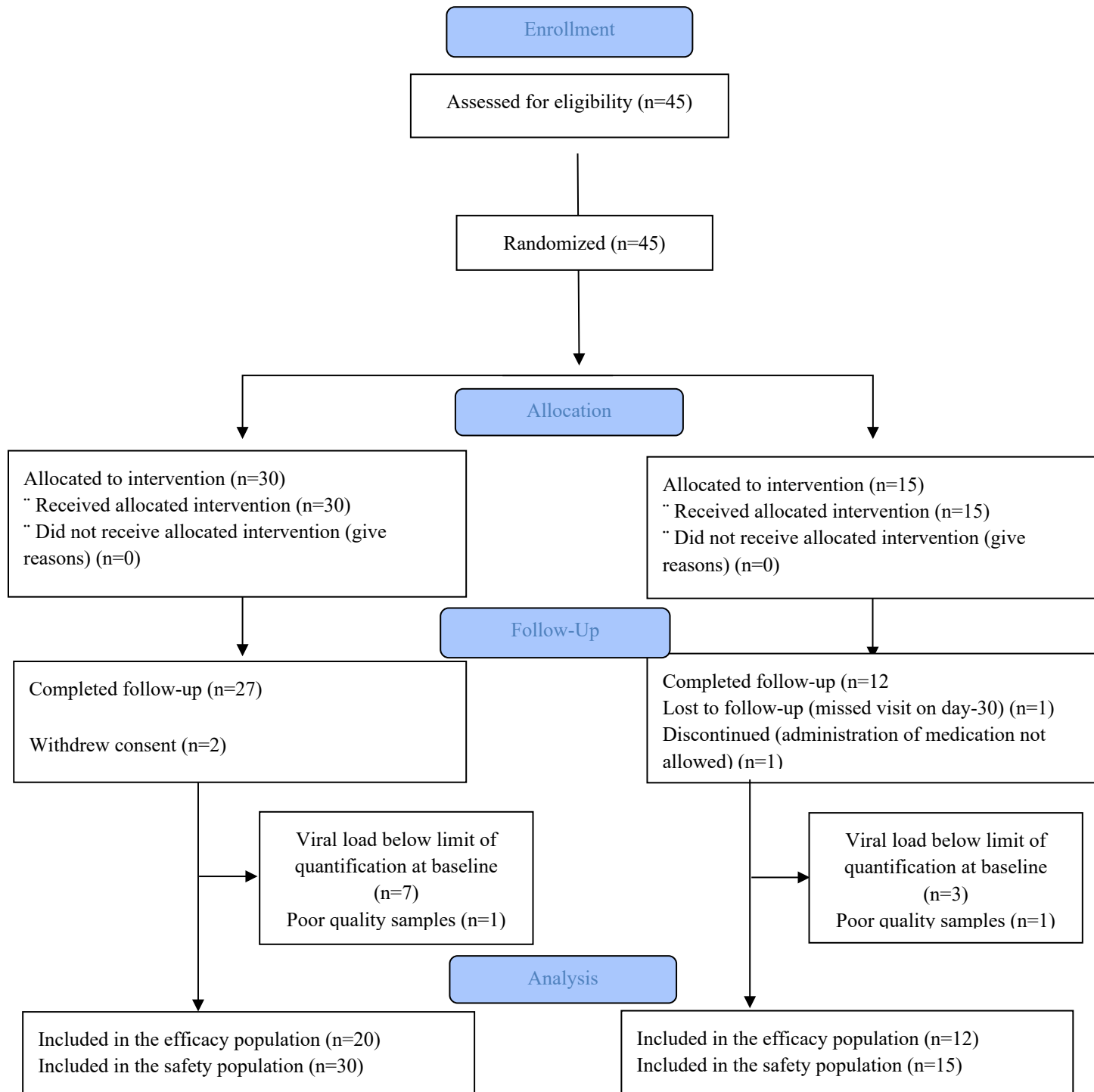
Baseline characteristics showed a mean age of 40·89 (SD 12·48) among study participants, without differences between groups; sex distribution was 20 (44%) females and 25 (56%) males (table 1). Comorbidities and disease stages were similar between groups; with the most frequent comorbidity being obesity in both groups, followed by hypertension, diabetes and chronic obstructive pulmonary disease (table 1). The number of days between symptoms onset and enrolment had a mean (SD) of 3·6 days (1·4) in the control group and 3·5 (1) in the treated group, without significant differences between groups nor cases enrolled beyond the eligibility criteria of up to 5 days since symptoms onset. No major differences in clinical symptoms, signs, or laboratory parameters were observed between groups at baseline (table 1). 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 between groups.

Quantitative viral load at baseline of the whole study population had a median (IQR) of 3·69 \log_{10} copies/reaction (1·97-5·74), including 5 (11%) undetectable cases and another 5 (11%) detectable although with <15 copies/reaction (<1·18 \log_{10} copies/reaction) at baseline, all these cases remaining undetectable in most samples through the follow-up and excluded from the efficacy analysis. No differences in viral load were detected between males and females. Another 4 cases including 2 with poor quality samples and 2 with early withdrawal were also excluded. The remaining 32 cases (20 treated and 12 control) constitute the efficacy analysis population (fig 1). Baseline viral load in this population had a median (IQR) of 5·59 \log_{10} copies/reaction (4·75 - 6·12) in the control group and 3·74 \log_{10} copies/reaction (2·8 - 5·79) in the treatment group ($p=0\cdot08$) (table 1). Viral load dynamics changed similarly in both groups over the study period without significant differences and decreasing over time (figure 2).

The estimation of IVM systemic exposure in treated patients was based on the collection of blood samples at 4-hours after each of the first 3 doses, after the 5th, and at 48 hours after the last

administered dose. This time-sampling design aimed at measuring the predicted IVM plasma peak plasma concentration (Cmax) and estimating the pattern of drug elimination from the body.

Figure 1: Trial profile



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

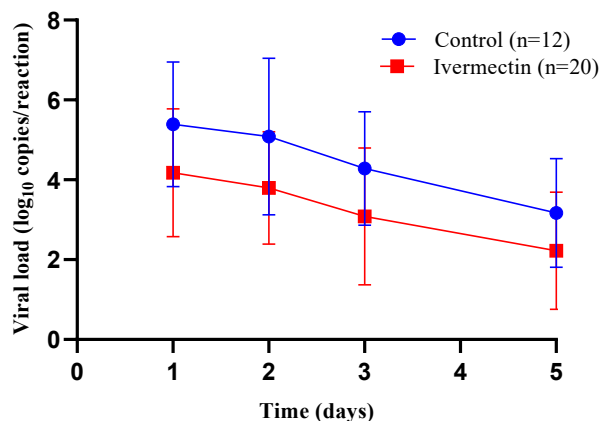
Table 1. Baseline characteristic of the study population

Numeric variables are reported as median (IQR)mean ± 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. No statistical differences were observed in any of the reported parameters between groups

Viral load reduction between baseline and day-5 was also similar between groups (figure 2). 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 ($r: 0.44$; $p<0.04$). This correlation was stronger when the reduction in viral load was related to the IVM exposure corrected by viral load at baseline ($r: 0.60$; $p<0.004$). The mean IVM plasma concentration levels also showed a positive correlation with the viral decay rate ($r:0.47$, $p=0.02$).

Figure 2. Viral load by quantitative RT-PCR on upper respiratory tract secretions since treatment initiation in patients receiving IVM 0.6 mg/kg/day for 5 days versus untreated controls. Data are mean (SD). Day-1 indicates



baseline measurements.

Based on the observed antiviral response, treated patients were divided in two subgroups where 160ng/ml was the threshold plasma concentration. Median C_{max} in this subgroup was 202 ng/ml (IQR: 167-268 ng/ml) in the >160ng/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 reductions between baseline and day-5 were analyzed in the 2 subgroups of IVM treated patients (figure 3a), with median (IQR) reductions in viral load of 42% (31-73) in the control group, 40% (21-46) in treated patients with <160ng/mL median plasma concentrations,

and 72% (59-77) in the higher concentration group (Kruskal-Wallis $p=0.0096$), with a statistically significant differences between the latter and the other groups (figure 3b).

Drug-induced effects on viral clearance were also assessed using viral decay rates as an endpoint parameter and its relationship with drug exposure. The viral load decay rate in treated patients with IVM plasma levels $>160\text{ng/mL}$ was significantly greater (median 0.64 d^{-1}) compared to that calculated in the untreated control group (median 0.13 d^{-1}) and also to the subgroup with $<160\text{ng/mL}$ median plasma concentrations (median 0.14 d^{-1}) ($p=0.041$) (figure 4a). Similar differences in decay rate were observed comparing controls and subgroups of patients with ratio $\text{AUC}_{\text{IVM}}/\text{AUC}_{\text{Vl}} >50$, and ratio $\text{AUC}_{\text{IVM}}/\text{AUC}_{\text{Vl}} < 50$ ($p=0.0006$) (figure 4b). No significant differences in baseline viral load were observed between IVM concentration subgroups. The relationship between IVM concentration did not correlate with body weight ($r^2=0.1$) or body mass index ($r^2=0.07$) among the 28 patients that completed treatment with IVM.

Adverse events were reported in 18 (40%) of the 45 patients included in the safety analysis, which by the design of the study was unblinded; 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) was reported in the trial 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.¹⁷ A relationship between IVM systemic concentration and adverse events failed to reveal a link between these variables.

Figure 3. Viral load by quantitative RT-PCR on upper respiratory tract secretions since treatment initiation (mean and SD) (A) and viral load reduction between baseline and day-5 (median and IQR) (B) in untreated controls and IVM treated patients discriminated by their median IVM plasma concentrations. All treated patients receiving IVM 0.6 mg/kg/day for 5 days. Day-1 indicates baseline measurements.

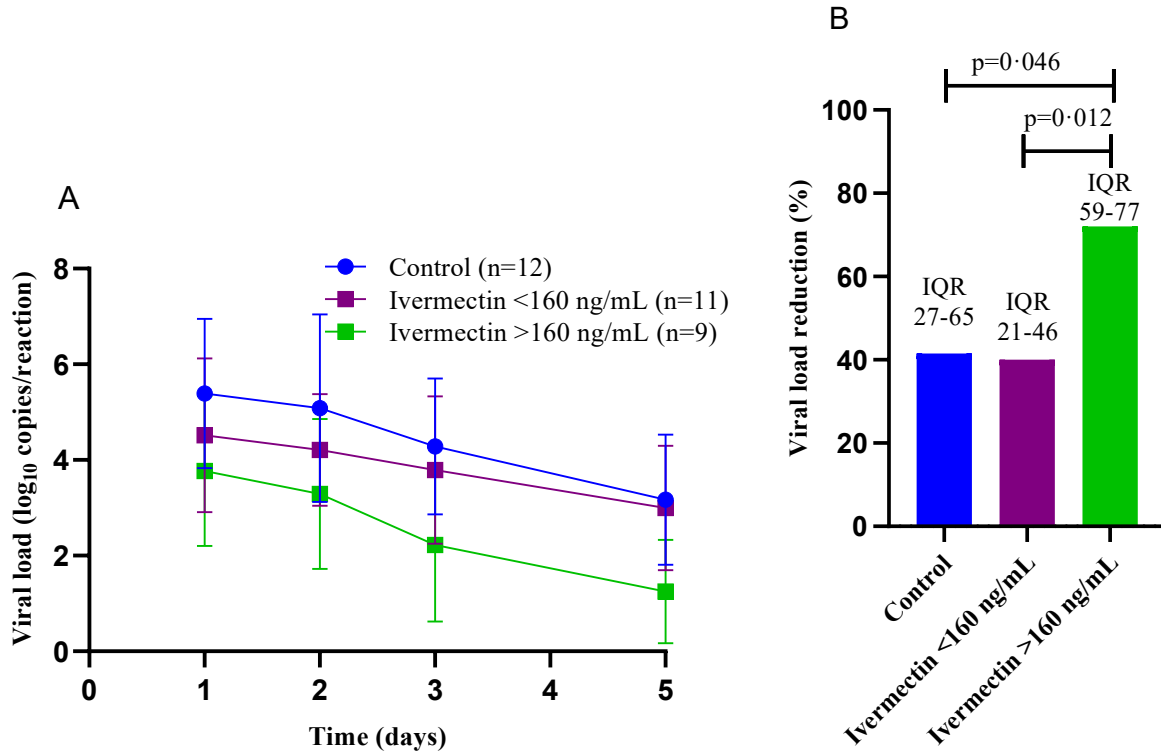
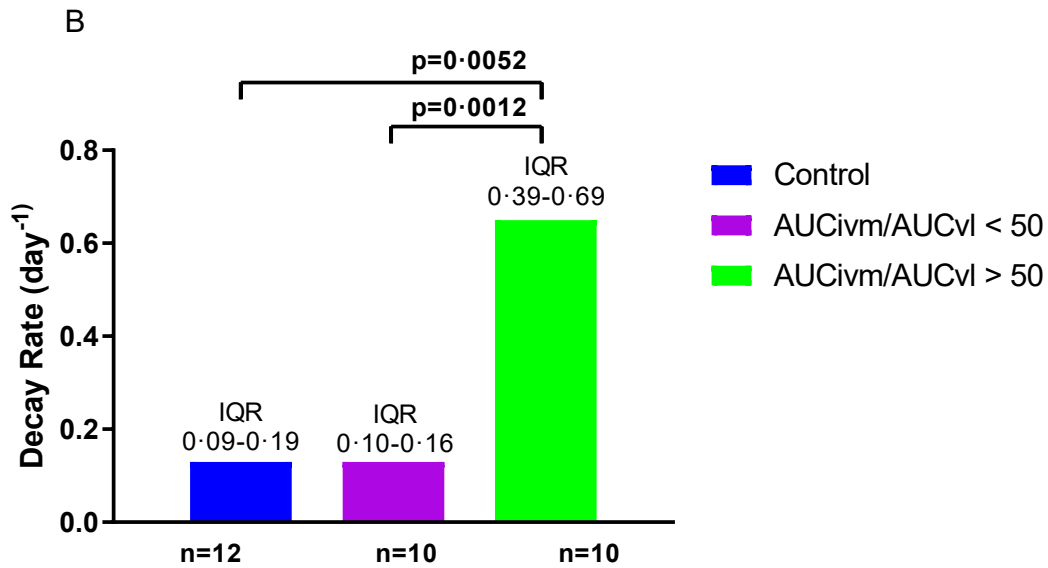
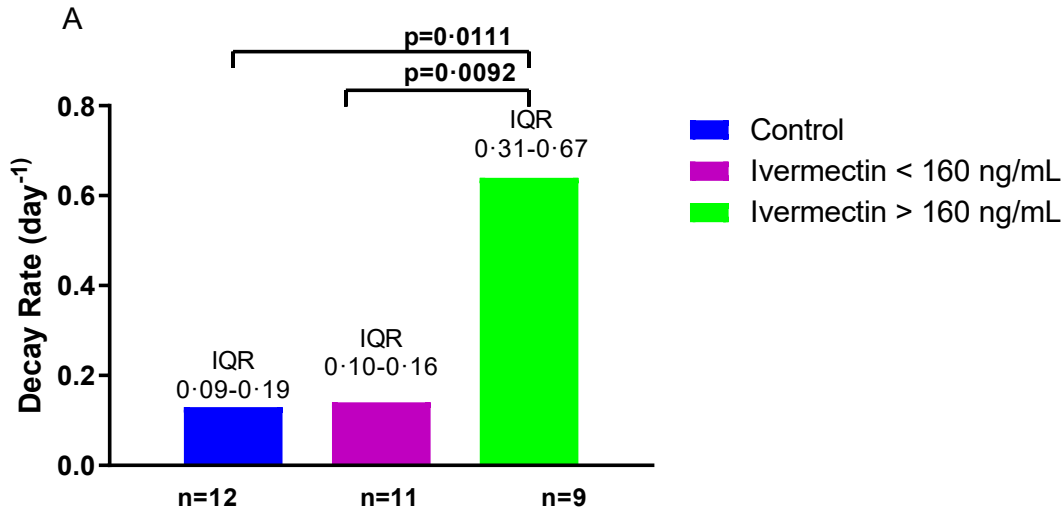


Figure 4. Viral load decay rates by quantitative RT-PCR on upper respiratory tract secretions in untreated controls and IVM treated patients according to median plasma concentrations of IVM (A) and by the ratio between the area under the IVM plasma concentration curve and the area under the viral load curve (AUC_{IVM}/AUC_{VL}) (B). Data are expressed as median (IQR).



	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**

Table 2. Summary of events in safety population. AE: adverse event. SAE: serious adverse event. *: hyponatremia; **: include the SAE (hyponatremia) and ALT and AST elevation, both in the same patient.

Discussion

The results of this pilot clinical trial indicate a concentration-dependent antiviral activity of IVM over SARS-CoV-2 infected patients with mild and moderate disease stages treated within 5 days of symptoms onset. This is the first clinical trial that confirms previous *in vitro* activity shown in Vero-hSLAM cell cultures.⁵ The relevance of IVM plasma concentrations as a surrogate indicator of drug exposure in the sites of viral replication such as nasopharyngeal mucosa and lung tissues, confirms theoretical models stating the need for higher doses than usual to achieve *in vivo* antiviral activity; however, contradict those concerns stating that those drug concentrations would not be achievable at doses with an adequate safety profile.^{6,18} A large IVM distribution into different tissues has been demonstrated in different animal species. The extensive pattern of IVM distribution to lung tissue has been well characterized in cattle with a lung tissue to plasma ratio of 2.67.¹² Considering that similar volumes of distribution have been reported for IVM in both cattle and humans and the systemic availability observed in treated patients 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.¹⁹

The beneficial antiviral effect was seen in the viral load reduction between baseline and day-5 and in viral decay rates only after IVM plasma concentration measurements allowed discrimination between patients achieving higher levels and identifying a strong direct relationship between drug systemic exposure and parameters of viral elimination. Using the threshold of 160 ng/mL for IVM plasma concentrations as an indicator of adequate systemic exposure, statistically significant differences compared to untreated controls were demonstrated even after adjusting for baseline viral load, with those falling below 160 ng/mL reaching viral elimination parameters similar to the control group. 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 was already extremely low or undetectable at baseline; a finding similar to what has been observed in another trials²⁰, highlighting the relevance of adequate timing of implementation of antiviral treatment, a critical issue in the design of clinical trials of potential antiviral therapies.

IVM plasma concentrations >160ng/mL were measured in 9 (45%) patients included in the efficacy analysis population. In another trial using a 3-day regimen of 0.6mg/kg for mosquitocidal activity and malaria control among adults, median C_{max} (CI95%) was 119ng/mL (45-455).⁸ Diet is a key variable affecting the oral bioavailability of IVM, with increased plasma concentrations achieved with fed state, mostly related to the fat content of the meal; still other variables probably play significant roles since IVM is characterized by a high intra and interindividual variability in key PK parameters.^{21,22} The interaction of IVM with ABC transporters as P-glycoprotein²³ and the modulation of P-glycoprotein activity after oral

administration is well known.²⁴ 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.

Safety and efficacy of high dose IVM are a subject of research in view of its potential role in increasing clinical usefulness in new indications as is the case of malaria and also aiming for simplified fix-dose rather than weight-based strategies as in Mass Drug Administration campaigns against different Neglected Tropical Diseases. Although further information is needed, this trial adds evidence on the safety of multiple-day high-dose regimens of IVM, without unexpected findings.

Limitations of this trial include a small sample size to detect clinical outcomes beyond antiviral activity, being the latter the critical question this trial was designed to answer. The finding of 2 distinct populations regarding mean IVM systemic concentrations was identified despite the body weight-based dosing and the indication of administering the drug tablets with meals. The lack of a proper registry of the actual content of the meals ingested around the intake of each treatment may add a source of variation to the observed IVM plasma profiles among treated patients.

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 short incubation periods, relatively rapid immune control of viral replication, and the high variability in symptom scores among patients.²⁵ For that reason, key components for adequate endpoints in these trials are sensitive quantifiable measurements of the underlying cause of disease as the quantitative RT-PCR with a wide dynamic range, to control high inter-individual variability in natural infection.²⁶ Still in this trial, the addition of an integrated assessment taking into consideration drug PK parameters was necessary to identify the potential clinical utility of IVM in SARS-CoV-2 infections. In IVM treated patients, AUC_{ivm}/AUC_{v1} ratios >50 were associated with significantly higher viral decay rates. 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 in antiviral activity.^{26,27} The potential clinical relevance of these findings remains to be confirmed in trials with clinical endpoints. Beyond clinical aspects of the illness, lowering viral burden might influence infectivity, although there is conflicting data regarding the relationship between burden of viral shedding and infectivity.²⁸

Drug repurposing has focused great attention on the search for viable treatments in the context of COVID-19 pandemic, allowing reduced development time and cost. In addition, the use of host-based antiviral compounds has been proposed as an alternative strategy over direct antivirals, that could overcome limitations derived from drug resistance or viral mutations.²⁹ The proposed antiviral mechanism of IVM is through its ability to inhibit the nuclear import of viral proteins

mediated by IMP α / β 1 heterodimer ⁵, and it has also been suggested that IVM could promote defense mechanisms such as pyroptosis in infected epithelial cells.³⁰

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 as either sole intervention or in combination with other tools for the control of the ongoing pandemic outbreak are warranted.

Contributors

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Declaration of interests

AK reports grants from Laboratorio Elea/Phoenix. MAT, MDG and ES are employees of Laboratorios Elea/Phoenix. SG is a member of the Board of Directors of Laboratorio Elea/Phoenix. All other authors declare no competing interests.

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.

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