Efficacy of the use of mefenamic acid combined with standard medical care vs. standard medical care alone for the treatment of COVID-19: A randomized double-blind placebo-controlled trial

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Abstract. Mefenamic acid is a non-steroidal anti-inflammatory drug exhibiting a wide range of anti-inflammatory, antipyretic, analgesic and probable antiviral activities. The present study evaluated the efficacy of treatment with mefenamic acid combined with standard medical care vs. standard medical care plus a placebo in ambulatory patients with coronavirus disease 2019 (COVID-19; nasal/oropharyngeal swabs reverse transcription-PCR test results positive for severe acute respiratory syndrome coronavirus 2). The present study is a phase II prospective, two-arm, parallel-group, randomized, double-blind placebo-controlled clinical trial which analyzed 36 patients. Two aspects were evaluated during the 14-day follow-up period: i) The time for reaching a patient acceptable symptom state (PASS), and ii) the last day of each COVID-19 symptom presentation. Adverse effects were evaluated. The clinical severity for all the patients in the study was mild (88.9%) and moderate (11.1%). The control (placebo) group achieved PASS on day 8.0±1.3, compared with day 4.4±0.8 in the mefenamic acid group (P=0.020, Kaplan-Meier analyses using log-rank tests). Patients that received mefenamic acid plus standard medical care had a ~16-fold higher probability of achieving PASS on day 8 (adjusted RR, 15.57; 95% CI, 1.22-198.71; P=0.035), compared with the placebo plus standard medical care group. All symptoms lasted for fewer days in the mefenamic acid group, compared with the placebo group; however, only the symptoms of headache (P=0.008), retro-orbital eye pain (P=0.049), and sore throat (P=0.029) exhibited statistically significant differences. The experimental treatment produced no severe adverse effects. On the whole, the present study demonstrates that the administration of mefenamic acid markedly reduced the symptomatology and time to reach PASS in ambulatory patients with COVID-19. Due to its probable antiviral effects and potent anti-inflammatory mechanisms, mefenamic acid may prove to be useful in the treatment of COVID-19, in combination with other drugs, including the new antivirals (remdesivir, molnupiravir, or favipiravir). However, future studies are also required to confirm these findings.

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Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has presented an unprecedented challenge to public health, with a substantial loss of human life worldwide (1). The rate of asymptomatic SARS-CoV-2 infection has been calculated to be between 28 and 31.4% (2). The spectrum of symptomatic infection ranges from mild to critical. The majority of patients

experience mild, self-limiting upper respiratory tract infection (~80% of symptomatic patients) (3,4) and only a small number of patients develop acute respiratory distress syndrome that can rapidly lead to multiorgan failure and death (5). A study conducted in Mexico reported that the most common initial symptoms are headache, fatigue, myalgia, arthralgia, fever, sore throat and cough (4); however, research conducted worldwide has reported a very heterogeneous clinical symptom frequency (6). Studies on the time needed by patients with COVID-19 to achieve a normal state of health (with standard medical care) have reported that 50-65% of patients regained their normal state of health 7 days from the date of diagnosis, whereas 35% of patients had not returned to their normal state of health 12-14 days after receiving a positive test result (4,7).

COVID-19 is an acute viral disease whose severity is associated with the dysregulation of inflammatory immune responses, which in turn inhibits the development of protective immunity against infection (8). The most severe complications of COVID-19 infection are sepsis-like inflammation, coagulopathy and respiratory or cardiovascular complications. The therapeutic strategies for the management of severe symptoms are focused on the control of viremia and/or inflammation, in addition to providing optimal organ support (8-14). The use of non-steroidal anti-inflammatory drugs (NSAIDs) for the treatment of COVID-19 was considered controversial at the onset of the pandemic, due to concerns that these types of drugs may increase the risk of infection or the severity of SARS-CoV-2 (15). However, the World Health Organization (WHO), as well as a recent meta-analysis, found no indication to date of any negative association between the use of NSAIDs, including ibuprofen, and SARS-CoV-2 infection or its outcomes (15). NSAIDs, such as ibuprofen, may prove beneficial for the early management of COVID-19 by ameliorating the suggested inflammatory process leading to lymphopenia and immunosuppression that has been reported to be a common feature associated with severe disease (16). Ibuprofen and diclofenac potassium have been shown to be superior to the currently used paracetamol, not only with regard to their analgesic and antipyretic effect, but also in significantly improving the lymphocytic count in patients with COVID-19, enhancing their immune response and favoring a 5-day recovery period (15,16).

Mefenamic acid is a NSAID that exhibits a wide range of anti-inflammatory, antipyretic and analgesic activities (17). In a previous study on a series of five cases, the early initiation of treatment with mefenamic acid provided symptomatic relief in reducing fever in non-hypoxic patients (18); in addition, the use of mefenamic acid led to reduced C-reactive protein (CRP) values, which can also prevent the cytokine storm, reflecting the significant anti-inflammatory activity of mefenamic acid in patients with COVID-19 (18). The use of mefenamic acid in post-COVID myalgia, until the CRP levels decrease to <1, has also been suggested (17,18). In addition, mefenamic acid exerts an inhibitory effect on the serine proteases of the RNA virus (DENV2 NS2B-NS3 protease of the dengue virus) (19). Various members of an expert panel in India shared their anecdotal experience on the effectiveness of mefenamic acid as an antipyretic, analgesic and anti-inflammatory agent in the management of COVID-19 (20). The expert panel recommended the use of mefenamic acid (500 mg, three times a day, for 7 days) for treating COVID-19 in adults; however, they stated that further extensive clinical trials are required to confirm the same findings in the management of COVID-19 (20).

Therefore, the present study was designed to randomly select patients with COVID-19 on an outpatient basis and compare the safety and efficacy of standard medical care combined with mefenamic acid, vs. standard medical care (mainly treatment with acetaminophen) (14) plus a placebo.

Patients and methods

Study design. A prospective, randomized, double-blind, two-arm, parallel group, phase II clinical trial was conducted between August and December, 2020, and was performed according to the 'CONSORT statement' guidelines for randomized controlled trials (http://www.consort-statement.org/). The present study was conducted to evaluate the safety and efficacy of mefenamic acid for decreasing the time period for patients to reach an acceptable symptom state [patient acceptable symptom state (PASS)]. It was approved by the Ethics Committee of the Mexican Social Security Institute, delegation of the State of Colima, Mexico (July 29, 2020), and written statements of informed consent were obtained from all the participants. The trial was performed in accordance with the principles of the Declaration of Helsinki and the international conference on harmonization-good clinical practice guidelines. The present clinical trial was registered as MEFECOVID-19: RPCEC00000388 in the Cuban Public Registry of Clinical Trials (RPCEC, the Spanish acronym) database (August 31, 2021).

Study subjects. The inclusion criteria were the following: Males and non-pregnant women ≥18 years of age, presenting with COVID-19 infection and a positive diagnosis of SARS-CoV-2 provided using reverse transcription PCR, that had a medical consultation due to their illness and were indicated for at-home ambulatory treatment. The women agreed to utilize effective contraceptive measures during the study period and for at least 15 days after the final drug administration of the analysis. The exclusion criteria comprised pregnant or breastfeeding women and patients presenting with any of the following conditions, prior to the diagnosis of COVID-19: Cancer, ischemic heart disease, chronic decompensated systemic disease, creatinine levels 1.25-fold higher than the normal values or a creatinine clearance <50 ml/min (Cockcroft-Gault method), blood hemoglobin levels <10 g/dl, drug addiction (use of illegal drugs), or any known liver disease with a doubling of liver function test values [aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, or bilirubin). Additionally, the following elimination criteria were used: Patients that voluntarily decided to abandon the study, patients that presented with severe toxicity [grade ≥3, according to the common terminology criteria for adverse events (CTCAE) v5.0, US Department of Health and Human Services (21)] attributable to the administration of the experimental drug, at some point of the study. No participant was vaccinated against SARS-CoV-2 prior to the development of the study, given that the vaccines were only available in Mexico after closing this clinical trial.

The physicians participating in the project identified candidates at the public secondary healthcare center, the Zone 1

General Hospital of the IMSS Colima, in the Mexican State of Colima, and requested their participation in the study. The patients that agreed to be included were randomly allocated to the experimental group (oral mefenamic acid + standard medical care) or the control group (placebo + standard medical care). Simple randomization was performed using computer-generated random allocation cards (groups A and B). All the patients were advised that they would continue to be under the supervision of their regular physician. The research team would not, under any circumstances, modify or limit any intervention that their usual physician, or they themselves, considered pertinent, such as going to the emergency department if there were alarm symptoms.

Intervention. All the patients received standard medical care and treatment prescribed by their family physician or specialist, which consisted of the administration of 500 mg oral paracetamol every 6-8 h, in addition to any other medication indicated by their treating physician (Table I). The experimental group was administered mefenamic acid (500 mg, Ponstan tablets, Pfizer, S.A. de C.V., Mexico) every 8 h for 7 days. The control group was administered a placebo starch tablet every 8 h for the same amount of time. The pills were recommended to be taken with milk or with meals, to reduce gastrointestinal adverse events. All patients (experimental and control groups) were administered one 20 mg omeprazole tablet daily during the study. All patients were instructed to visit the emergency service if they presented with respiratory difficulty or worsening of symptomatology. The researchers did not intervene in drug prescription or lifestyle indications.

Outcome measures and follow-up. There were two co-primary endpoints. The first primary endpoint was the time for reaching PASS, defined as the value of symptoms the patient considered to be wellbeing thresholds of pain and function. The study incorporated the most widely used anchoring question to identify PASS cut-off points, which was the following: 'Taking into account all your daily activities, do you consider your current state satisfactory, in relation to pain level and functional impairment?' The response options were 'Yes' or 'No' (4). Treatment success was defined as a PASS answered in the affirmative on days 1-14 of follow-up.

The second endpoint was the last day the patient presented with each symptom of COVID-19. Fever, fatigue, arthralgia, myalgia, headache, sore throat, nausea, vomiting, diarrhea, dizziness, conjunctivitis, rhinorrhea, exanthema, skin rash, and loss of sense of smell or taste were catalogued as present or absent for each day of follow-up. Adverse events were monitored by the researchers through anamnesis. Follow-up was carried out for at least 14 days or until patient outcome (cure or death). Daily follow-up was suspended in the hospitalized patients. From the first day of hospital admission, their registers were considered lost data and were not considered in the analysis from that day forward. The exception was the PASS, and its subsequent registers were reported as a negative acceptable symptom state. Nevertheless, the general aspects of hospitalization and outcome (cure or death) of those patients were registered.

The patients also had a baseline disease severity classification of mild, moderate, severe, or critical disease, as previously described and as directed by the WHO interim clinical management guidance (22,23). In addition, the concepts of asymptomatic patients (0 major symptoms and 0 minor symptoms) and pauci-symptomatic patients (0 major symptoms and 1-2 minor symptoms) were considered, as previously defined (major symptoms: Fever >37.8˚C and new persistent cough; minor symptoms: Hoarseness, non-persistent cough, sore throat, runny or stuffy nose, shortness of breath, wheezing, headache, muscle aches, nausea and/or vomiting and/or diarrhea, and loss of sense of taste or smell) (24). The patients were also evaluated using a severity score, which was determined by the response to the question: 'Considering all the ways in which illness and health conditions may affect you at this time, please indicate below how you are doing?' The response options were measured on the 0-10 visual analogue scale (VAS), from 'very well' (score of 0) to 'very poorly' (score of 10), as previously described (4). That question is validated in the routine assessment of patient index data 3 (RAPID3) (25), previously used on non-hospitalized patients with COVID-19, in which 0 indicates 'no symptoms' and 10 indicates 'severe symptoms' (4).

Blinding. The patients, the clinicians evaluating them, and the physicians that performed the statistical analyses were blinded to the assigned therapy group.

Sample size. The sample size calculation was based on the number of patients that had a PASS on day 8. It was estimated that 95% of the patients in the experimental group and 56% of the controls would reach a PASS on day 8, based on previously published data that described achieving a PASS on day 5 of treatment in mild COVID-19 (4). A total of 36 patients were needed to reach the required power (0.8), when the statistical analysis was performed at the level of the two-tailed alpha value (0.05) (26). At the end of the study, the statistical power for detecting a difference between two distinct groups was calculated, utilizing the number of patients with a PASS on day 8, resulting in 84.7% (26).

Statistical analysis. Data are presented as the mean ± standard deviation (for data with normal distribution), and as median with interquartile range (IQR) for data with a non-normal distribution or percentages. Normal data distribution was first determined using the Kolmogorov-Smirnov test, and the equality of variances was confirmed using Levene's test. Numerical data with normal distribution (e.g., body mass index or age) were compared between groups, utilizing an unpaired Student's t-test, whereas ordinal data were compared using the Mann-Whitney U test. Categorical values were compared using Fisher's exact test or the likelihood ratio Chi-squared test. Kaplan-Meier analyses were performed with the use of log-rank tests. Binary logistic regression analyses were employed to determine the probability of achieving a PASS on day 8 (binomial outcome of yes or no), in the mefenamic acid group, compared with the placebo group. Data were summarized as relative risks (RRs) with 95% confidence intervals (CIs) and P-values, adjusted for age, sex, obesity, diabetes, hypertension, progression time and baseline severity.

Statistical analysis was carried out using SPSS version 20 software (IBM Corp.), with the exception of the number needed

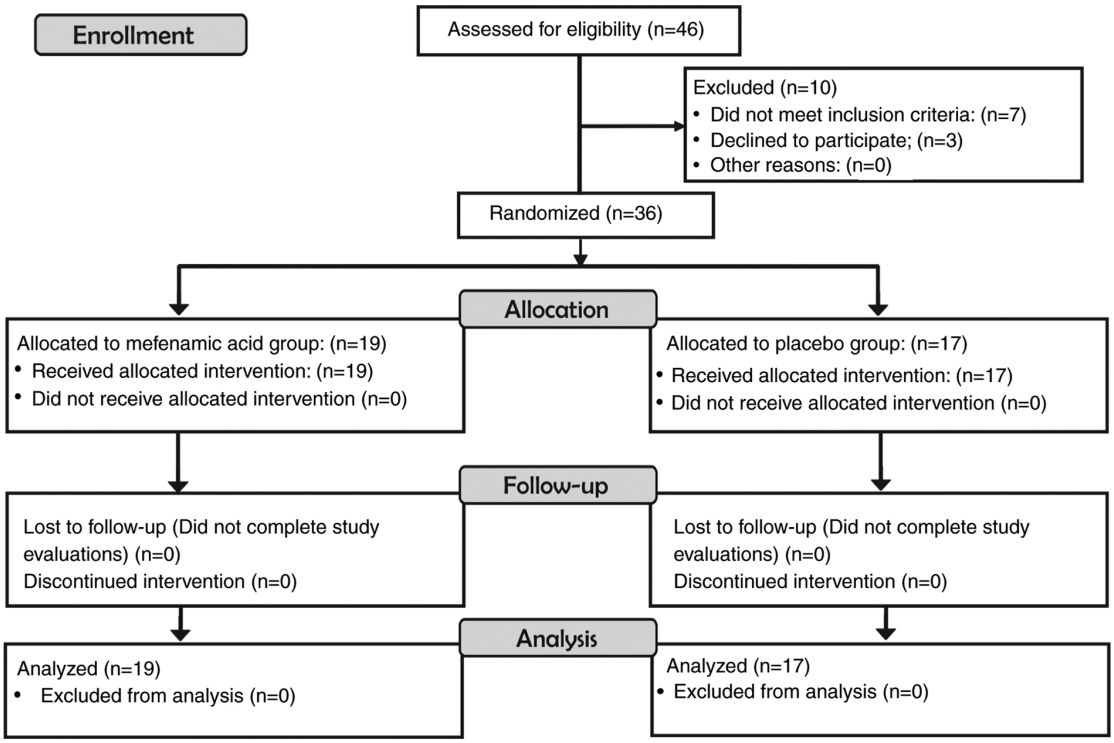

Figure 1. CONSORT 2010 flow diagram displaying the number of patients screened, included, eliminated and analyzed.

to treat (NNT), which was calculated using MedCalc v17.7.2 software (MedCalc Software Bvba), and sample size and statistical power, which were calculated using online ClinCalc.com software (ClinCalc LLC), to compare two proportions: Two-sample, two-sided (26). A value of $P<0.05$ was considered to indicate a statistically significant difference. Sample size and statistical power were calculated for a one-tailed test. The remainder of the analyses were two-tailed tests.

Results

A total of 36 patients were randomized and analyzed. A total of 19 patients in the experimental group and 17 patients in the control group agreed to participate in the study. No patient discontinued the intervention (Fig. 1).

The clinical severity of SARS-CoV-2 infection for all the patients in the present study was mild (n=32, 88.9%) and moderate (n=4, 11.1%), according to the WHO interim clinical management guidance (22,23). The symptom severity score for all the patients, according to a self-assessment 10-point visual analogue scale, was 6.5±2.7, whereas the reported median number of COVID-19-compatible symptoms was 11.5 (IQR, 5). None of the patients were asymptomatic or pauci-symptomatic as all required therapy under the guidance of a healthcare professional. Even though the patients had no clinical or imaging signs suggestive of pneumonia, they were very symptomatic. The main clinical characteristics and prescribed drugs are listed in Table I, together with the homogeneous characteristics between the groups (experimental vs. control) at the beginning of the study (Table I).

The control group achieved a PASS on day 8.0±1.3, compared with day 4.4±0.8 in the experimental group (P=0.020, derived from Kaplan-Meier analyses using log-rank tests) (Table II and Fig. 2). The univariate and multivariate analyses revealed that patients receiving mefenamic acid plus standard medical care had an ~16-fold higher probability of achieving PASS on day 8 (adjusted RR, 15.57; 95% CI, 1.22-198.71; P=0.035), compared with the standard medical care plus placebo group (Table III). For 1 patient to achieve the benefit of PASS on day 8 or earlier, the number of patients needed to be treated with mefenamic acid was 2 patients (NNT 2.4; 95% CI, 1.50-5.89; P=0.029). Only 1 patient required hospitalization and there were no deaths, with no statistically significant differences between the groups, regarding those parameters (Table III).

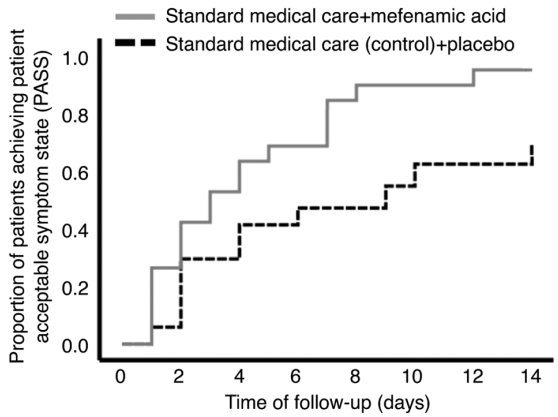

Figure 2. Kaplan-Meier curves illustrating patient progression. The group of patients that received mefenamic acid achieved a patient acceptable symptom state in fewer days, compared with the patients that received placebo. Both groups also received standard medical care (4.4±0.8 vs. 8.0±1.3, respectively, P=0.020). The results of the statistical analysis for these data are presented in Table II. Kaplan-Meier analyses were performed with the use of log-rank tests.

Table I. Main clinical characteristics of the participating subjects at the moment of enrollment and standard prescribed drugs.

Clinical characteristic	All (n=36)	Control (n=17)	Experimental (n=19)	P-value
Women, n (%)	24 (66.7)	11 (64.7)	13 (68.4)	0.546[a]
Age (years), n (%)	39.5±15.4	42.5±15.9	36.8±14.8	0.276[b]
>60 years old, n (%)	6 (16.7)	3 (17.6)	3 (15.8)	0.614[a]
Body mass index	30.2±6.8	31.2±8.5	29.3±5.0	0.402[b]
Obesity, n (%)	17 (47.2)	7 (41.2)	10 (52.6)	0.363[a]
Diabetes, n (%)	2 (5.6)	1 (5.9)	1 (5.3)	0.729[a]
High blood pressure, n (%)	3 (8.3)	1 (5.9)	2 (10.5)	0.543[a]
Asthma, n (%)	1 (2.8)	0 (0.0)	1 (5.3)	0.543[a]
Smoking, n (%)	1 (2.8)	1 (5.9%)	0 (0.0)	0.472[a]
Progression time[e]	3.2±2.2	2.6±1.7	3.7±2.5	0.127[b]
Body temperature (˚C)	36.9±0.8	37.0±1.0	36.7±0.6	0.228[b]
% SpO_2	96.3±2.0	96.4±1.6	96.2±2.3	0.831[b]
SpO_2 <94%, n (%)	6 (16.7)	2 (11.8)	4 (21.1)	0.386[a]
Degree of dyspnea	1.0±1.8	1.3±1.7	0.8±1.7	0.244[c]
Symptom severity[f]	6.5±2.7	6.5±2.6	6.6±2.8	0.907[c]
No. of symptoms[g]	11.5 (5.0)	11.7 (4.0)	11.0 (4.0)	0.468[b]
Disease severity (WHO)[h], n (%)				0.655[d]
Mild	32 (88.9)	15 (88.2)	17 (89.5)	
Moderate	4 (11.1)	2 (11.8)	2 (10.5)	
Severe	0 (0.0)	0 (0.0)	0 (0.0)	
Number of drugs used for COVID-19[i]	1.7±1.2	1.7±1.3	1.6±1.1	0.743[b]
Paracetamol, n (%)	36 (100)	17 (100.0)	19 (100.0)	0.957[a]
NSAIDs[j], n (%)	4 (11.1)	2 (11.8)	2 (10.5)	0.906[a]
Ivermectin, n (%)	3 (8.3)	1 (5.9)	2 (10.5)	0.619[a]
Antibiotics, n (%)	4 (11.1)	2 (11.8)	2 (10.5)	0.906[a]
Antivirals, n (%)	2 (5.6)	1 (5.9)	1 (5.3)	0.935[a]
Steroids, n (%)	1 (2.8)	1 (5.9)	0 (0.0)	0.759[a]
Vitamins, n (%)	2 (5.6)	1 (5.9)	1 (5.3)	0.935[a]
Mucolitic, n (%)	2 (5.6)	1 (5.9)	1 (5.3)	0.935[a]

Percentages or averages and standard deviation are shown. [a]Data were analyzed using Fisher's exact test. [b]Data were analyzed using the Student's t-test. [c]Data were analyzed using the Mann-Whitney U test. [d]Data were analyzed using the likelihood ratio Chi-squared test. [e]Days from the appearance of the first symptoms. [f]Symptom severity score (patient overall self-assessment) using a 10-point visual analog scale (VAS). [g]Number of COVID-19-compatible symptoms, showing the median and interquartile range (IQR). [h]Disease severity was calculated as directed by the World Health Organization (23). [i]Number of drugs used for COVID-19, without considering prescribed drugs due to clinical trial (mefenamic acid, omeprazole, or placebo), the mean and standard deviation are shown. [j]Other NSAIDs used in addition to mefenamic acid included metamizole (administered to one patient in each group), aspirin (administered to one patient in the experimental group), and naproxen (administered to one patient in the control group). Antivirals include oseltamivir or amantadine; antibiotics include azithromycin, clarithromycin, or levofloxacin. SpO_2, oxygen saturation (determined using a pulse oximeter on the right-hand middle finger); the degree of dyspnea was evaluated with the Borg scale. NSAIDs, non-steroidal anti-inflammatory drugs.

The results with respect to the last day average of the symptoms analyzed per group are presented in Table IV. All the symptoms lasted fewer days in the mefenamic acid group, compared with the placebo group (Table IV). However, as regards the symptoms, the difference was statistically significant only in relation to headache, retro-orbital eye pain and sore throat. Myalgia, dizziness and rhinorrhea exhibited close to statistically significant values (Table IV).

As regards possible adverse effects related to mefenamic acid, 2 (10.5%) patients presented with abdominal pain/discomfort (grade 1 or 2 gastritis) at some point during the follow-up; however, symptomatology ceased after emphasizing that the patients take the medication

Table II. COVID-19 outcomes in the experimental and control groups.

Outcome	Control (n=17)	95% CI	Experimental (n=19)	95% CI	P-value[a]
Days for PASS	8.0±1.3	5.4-10.7	4.4±0.8	2.5-5.4	0.020
PASS on day 8	52.9%		94.7%		0.005
Hospitalized	0.0%		5.3%		0.528
Death	0.0%		0.0%		-

The control group received standard medical care plus placebo, and the experimental group received standard medical care plus mefenamic acid. [a]All values were compared using Fisher's exact test, apart from the days for PASS, which were compared using Kaplan-Meier analysis with log-rank tests. Days for PASS, the number of days for the patient to reach an acceptable symptom state (mean ± standard deviation); PASS on day 8, achieving acceptable symptom state on day 8; 95% CI, 95% confidence interval.

Table III. Relative risk ratio values for reaching PASS on day 8 in patients with COVID-19 treated with mefenamic acid, compared with the placebo (control group).

		95% CI		
PASS on day 8	Value	Lower	Upper	P-value
Crude RR[a]	16.00	1.72	148.43	0.015
Adjusted RR[b]	15.57	1.22	198.71	0.035
NNT[c]	2.4	1.50	5.89	0.029

The control group received standard medical care plus placebo, and the experimental group received standard medical care plus mefenamic acid. [a]RR, relative risk with 95% CI and P-value, calculated using binary-binomial-logistic regression analyses; [b]adjusted for the covariates of sex, age, disease severity (as directed by the World Health Organization), obesity, diabetes, high blood pressure, and the number of days from the appearance of the first symptoms in the patients to their inclusion in the study; [c]NNT, number needed to treat.

with meals. All the patients were administered omeprazole to prevent severe acute NSAID-related gastroduodenal damage. No laboratory tests were indicated by the patients' treating physicians during the follow-up. No patient required definitive suspension of mefenamic acid due to adverse effects.

Discussion

In the present study, in ambulatory patients with COVID-19 under standard medical care, the additional administration of mefenamic acid reduced the duration of the symptomatic illness, compared with ambulatory patients treated with standard medical care plus the placebo. Various signs and symptoms, such as headache, sore throat and retro-orbital eye pain improved at a significantly more rapid rate with the addition of mefenamic acid to standard medical care, reducing the time to reach PASS (8.0±1.3 to 4.4±0.8 days, P=0.020) (Tables II-IV and Fig. 2).

The beneficial effects of the administration of mefenamic acid can be generally associated with several mechanisms. The first is symptom reduction due to its antipyretic and analgesic activities. The reduction of inflammatory processes is another important effect. Based on the pathogenesis of COVID-19, NSAIDs have been suggested to be beneficial for the early management of COVID-19, by possibly ameliorating the inflammatory process leading to lymphopenia and immunosuppression (10), preventing disease progression, or even reversing lymphocytopenia.

Progression in patients with COVID-19 has been associated with the presence of the 'cytokine storm' induced by the virus and the hyper-inflammatory immune response of the host. One of the most critical pro-inflammatory cytokines of the innate immune response is IL-6. Mefenamic acid has been shown to exert an inhibitory effect on IL-6 levels (27), which may be beneficial for reducing disease progression.

The large majority of NSAIDs owe their anti-inflammatory effects to the inhibition of prostaglandin synthesis via cyclooxygenase (COX). Mefenamic acid has also another anti-inflammatory mechanism: Nucleotide-binding oligomerization domain-like receptor containing pyrin domain 3 (NLRP3) inflammasome activation intensely induces cytokine production as an inflammatory response to viral infection (28). Therefore, the NLRP3 inflammasome may be a potential target for the treatment of COVID-19. The NLRP3 inflammasome is responsible for processing the pro-inflammatory cytokine, IL-1β. Mefenamic acid has been shown to selectively inhibit the NLRP3 inflammasome and the release of IL-1β, independent of its COX-mediated anti-inflammatory activity (20,29).

It has also been demonstrated that mefenamic acid exerts a possible antiviral effect. In a previous study, *in vitro* antiviral activity against the Chikungunya virus (CHIKV) was observed, enhancing the effects of other antivirals, such as ribavirin (30). Its antiviral activity has been suggested to be

Table IV. Last day with the presence of different signs and symptoms of COVID-19 in the control and experimental groups.

	Last day with the sign or symptom				
Symptom	Control (n=17)	90% CI	Experimental (n=19)	90% CI	P-value[a]
Headache	8.8±4.9	6.6-11.1	4.7±4.4	3.0-6.5	0.008
Fatigue	10.8±5.5	8.3-13.2	9.7±4.2	7.9-11.4	0.250
Myalgia	6.3±2.5	4.1-8.4	3.9±3.3	2.4-5.3	0.065
Sore throat	4.6±4.2	2.7-6.5	2.3±1.4	1.7-2.9	0.029
Cough	8.4±4.6	6.4-10.4	8.3±5.1	6.0-10.5	0.471
RO eye pain	4.6±4.2	3.0-6.2	2.3±1.4	0.8-4.1	0.049
Arthralgia	4.9±4.4	2.8-7.0	4.0±4.2	2.2-5.7	0.277
Fever	2.0±1.2	0.9-3.0	2.3±2.1	0.6-4.0	0.375
Chills	3.1±3.1	1.2-5.0	2.1±1.4	1.2-3.0	0.213
Rhinorrhea	7.6±5.4	5.0-10.1	4.4±5.3	1.3-7.4	0.082
Nausea	6.2±4.5	3.7-8.7	5.4±3.7	3.4-7.5	0.344
Conjunctivitis	5.5±3.5	2.6-8.4	4.5±6.1	0.1-8.9	0.375
Anosmia	11.0±4.1	8.7-13.2	10.9±4.2	8.5-13.3	0.478
Ageusia	5.1±3.6	8.4-13.0	3.6±3.2	5.9-11.7	0.184
Dizziness	6.5±4.3	4.1-8.6	3.7±4.1	1.2-6.2	0.083
Vomiting	2.0±1.0	0.3-3.7	2.0±0.8	1.0-2.9	0.500
Diarrhea	5.1±1.0	3.2-7.1	3.6±3.2	1.5-5.7	0.168

The control group received standard medical care plus the placebo, and the experimental group received standard al medical care plus mefenamic acid. [a]Data were analyzed using a one-tailed t-test. Means ± standard deviations are shown. 90% CI, 90% confidence interval; RO eye pain, retro-orbital eye pain.

due to interactions with the viral envelope that lead to the inactivation of the virus, interfering with its internalization in Vero cells. Such an effect was not observed with other NSAIDs, including aspirin (30). In addition, mefenamic acid was previously shown to exert an inhibitory effect on the serine proteases of an RNA virus (DENV2 NS2B-NS3 protease of the dengue virus) (19). That antiviral effect was observed at a concentration of 20-30 mcM (4.8-7.2 mcg/ml) in the *in vitro* trials, and even at lower concentrations, including 5 mcM (1.2 mcg/ml), when combined with ribavirin (19,30). In *in vivo* experiments (CHIKV-infected mice), the antiviral effect of mefenamic acid was observed at a dose of 15 mg/kg (30). To provide context, a maximum plasma concentration of 3.6-5.9 mcg/ml has been calculated to be reached in an adult receiving 250 mg oral mefenamic acid, varying in each individual and depending on the formulation employed (31). At an oral dose of 1 g administered to an adult, the maximum plasma concentration has been reported at up to 10 mcg/ml (32). Of note, in the present clinical trial, the dose employed for patients with COVID-19 was 500 mg, administered three times a day, for 7 days. Thus, a probable antiviral effect of mefenamic acid after customary doses of the drug may be reached, albeit further studies are required to confirm the present findings. Previous in silico analyses have demonstrated that mefenamic acid is able to interact with the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) amino acid residues via a predominant metal coordination bond and hydrogen bonding with the active site (33). RdRp is a target protein in SARS-CoV-2 that has been validated and extensively studied for drug development, with respect to COVID-19 (33). Given that mefenamic acid has been shown to potentiate the effect of antiviral drugs, such as ribavirin (30), perhaps it could enhance the effect of antivirals used against SARS-CoV-2, such as remdesivir, molnupiravir or favipiravir (34).

Mefenamic acid is often used in the treatment of dysmenorrhea and heavy menstrual bleeding, at an oral dose of 1.5 g per day for 3-5 days; however, it has also been administered for prolonged periods of up to 6 months (35,36). The drug is generally well-tolerated. The most common adverse effects are related to symptoms of gastritis. Therefore, taking the medication with meals, and receiving omeprazole throughout the study, was a prime necessity. Contraindications for and precautions regarding its use should be evaluated in each patient, adhering to the widely described recommendations in the medical literature (37).

The present study has several limitations. All the patients included in the study were unvaccinated and the majority had mild COVID-19; therefore, other studies on patients with severe forms of the disease or vaccinated patients are warranted. Of note however, disease presentation in the majority of symptomatic patients with COVID-19 is mild or moderate; thus, the results of the present study are very relevant for community use. The sample size in the present study was sufficient and had adequate statistical power to determine the efficacy of mefenamic acid for more rapidly reaching an acceptable symptom state. However, an analysis with a greater number of patients and a follow-up including laboratory parameters, or serial respiratory viral load (to confirm the possible antiviral effect

of mefenamic acid), is recommended in future investigations. Notably, other NSAIDs, such as ibuprofen and diclofenac, have been shown to be superior to the currently used paracetamol, in patients with mild COVID-19 infection (15,16). Thus, further studies comparing the effects of various NSAIDs are required.

In conclusion, the present study demonstrated that the administration of mefenamic acid markedly reduced symptomatology and the time to reach PASS in ambulatory patients with COVID-19. Its administration was well-tolerated and there were no notable adverse effects. Given its probable antiviral effects and potent anti-inflammatory mechanisms, it may prove to be useful in the treatment of COVID-19, in combination with other drugs, including the new antivirals, such as remdesivir, molnupiravir or favipiravir (34). Nevertheless, future studies are required to analyze these aspects.

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Availability of data and materials

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Authors' contributions

JGE, IDE, HRGS, IPRS, JDE and MLMF designed the study and wrote the manuscript. HPGS, ACCV, JAGS, KAMR, CBCA, MWG, OBG, OGDE, HSGG and DCC visited and evaluated the patients. BAPM, FRL, VM and IDE designed and performed the statistical analysis. AGS and EMZ authorized and coordinated the recruitment of patients in the hospital. JDE and OGDE were the administrative coordinators of the clinical trial. IDE and HPGS confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Mexican Social Security Institute, delegation of the State of Colima, Mexico (July 29, 2020), and written informed consent was obtained from all the participants. All procedures performed in the present protocol were carried out in accordance with the Declaration of Helsinki and the clinical trial was registered as MEFECOVID-19: RPCEC00000388 in the Cuban Public Registry of Clinical Trials (RPCEC) database (August 31, 2021).

Patient consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

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